LABORATORY AND FIELD MANUAL OF BOTANY

BERGEN AND DAVIS

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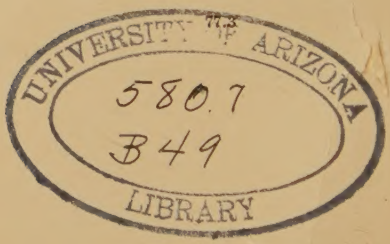

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PREFACE

This manual offers material for much more than a year's laboratory work. This is made necessary by the fact that instructors differ widely in their views as to what matter should be presented in an introductory course under the variety of conditions obtaining where botany is taught. A course must necessarily be framed selectively, and the chief alternatives are discussed in the opening paragraphs of the Introduction.

The authors fully recognize the fact that no set of directions of only moderate fullness can tell the student all that he needs to know about choice of material, apparatus, and manipulation. It is assumed that much is left to be explained by the instructor, and constant mention is made of general and special laboratory guides which may be consulted for needed details.

The student in the laboratory is not to consider himself as merely the corroborator of facts already ascertained: he is to interrogate mainly not the instructor, not the manual, but the plant itself. The directions here given are, therefore, for the most part suggestions on methods of procedure and indications as to the plants or parts of plants in which to look for desired information.

Since the amount of ground that can be covered by laboratory divisions varies so largely with many circumstances, it has seemed desirable to designate two courses, a briefer and a fuller one. The matter which may be omitted from the latter to frame the shorter course is printed in smaller type and consists in the main of rather more difficult or detailed studies than those which appear in the larger type. In a general way the order of treatment follows that of the authors' *Principles of Botany*, but the

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shorter course does not cover many more topics than are dealt with in Bergen's *Elements* or *Foundations of Botany*, and may be used with either of those books.

Part I consists mainly of studies on the gross anatomy and the histology of seed plants, together with a set of separately numbered experiments to illustrate some of the main principles of plant physiology.

Part II deals with type studies of spore plants, outlining the evolution and classification of the plant kingdom. Here will also be found studies on the gametophyte phases and the life histories of seed plants to show their relationships to the spore plants. Part II is introduced by outlines on the plant cell to illustrate the chief principles of growth and reproduction.

Part III is concerned with a series of laboratory and field studies which may serve to offer at least an outline for the treatment of ecology as a scientific subject. Profound ecological studies demand far more knowledge of taxonomy, plant physiology, meteorology, the physics and chemistry of soils, and kindred subjects than can be required of beginners in botany. However, the authors believe that it is quite possible to illustrate, even to beginners, something of the kind of quantitative discussion of variations in environment and the responses of plants to changed conditions, which must distinguish the ecology of the future.

Hearty acknowledgments for valuable suggestions are due to A. T. Bell, F. E. Clements, W. N. Clute, W. F. Ganong, B. Gruenberg, Miss Lillian J. MacRae, G. J. Peirce, and R. B. Wylie, who have wholly or in part read the manuscript or the proofs.

J. Y. B.
B. M. D.

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LABORATORY AND FIELD MANUAL OF BOTANY

INTRODUCTION

It is intended that these laboratory outlines shall be found adaptable to several methods of approach in framing a general course in elementary botany.

First. By beginning with Part I the student may consider first the more general features of the morphology of the seed plant and the most important of its physiological activities. This may then be followed by studies of a series of spore plants (Part II), to outline the chief steps in plant evolution. Such work as is possible in plant ecology (Part III) is thus deferred to the end of the course.

Second. By commencing with Part II the student will be introduced at once to the principles of cell structure, growth, and reproduction, and can then trace the evolution of the plant kingdom. By this arrangement selections from Part I will follow the studies of Part II, and Part III will receive attention last.

Third. Part I may be followed at once by Part III, and the studies of Part II be used only to illustrate such types and topics concerned with spore plants as may seem desirable.

Fourth. It is by no means necessary that the matter of Part I be taken up in the order given. Instead of beginning with the plant as a whole or with the seed, a course may be readily shaped so as to commence with the fruit or with the leaf.

The planning of a course depends upon so many factors, such as season, material, equipment, maturity of students, and the time

at the disposal of the class, that it must vary greatly with the different conditions. The authors, recognizing these difficulties, have tried to present a flexible outline in a thoroughly practical manual containing sufficient material to permit of a wide range of choice. In general they believe that the best results will be obtained, when a full year can be devoted to the subject, by taking the matter in the order given in the *first* or *second* of the alternatives presented above. If only a half year is available, the best course in their judgment is that indicated in the *third* alternative.

For the guidance of any who may care for such suggestions the authors have designated by double asterisks (* *) *those experiments and studies which they consider to be the most valuable.*

A brief discussion of laboratory methods and equipment is presented immediately before the laboratory outlines and experiments of Parts I, II, and III. It is hoped that the instructor may find some helpful suggestions in this, and certain parts are written expressly to aid the student to an understanding of the spirit of laboratory work, methods of drawing, note taking, and the care of instruments.

The essential methods of botanical microtechnique and the preparation of the material are taken up after the laboratory outlines. This account has been introduced to assist the instructor and the advanced student in the collection and preservation of material and in the more detailed studies of plant histology and cytology, which demand the preparation of microtome sections and critical staining methods. The discussion does not attempt to give such details covering special studies as may be found in several more exhaustive treatises to which the reader will be referred. It endeavors rather to outline standard methods of killing, fixing, preserving, cutting, and staining plant structures, which cannot fail to give good results, with the reasons why they have been selected. Some simple directions for the culture of algæ, fungi, moss protonema, fern prothallia, etc., follow the account of microtechnique.

A section entitled "Material, Apparatus, and Supplies" gives lists of preparations for the microscope, favorable material for histological work, apparatus and supplies, with the addresses of dealers who furnish these to the trade.

The bibliography has been chosen with the purpose of presenting a group of books many of which are within the possibilities even of a well-equipped school library, rather than a lengthy list of detailed literature which is usually only handled by the specialist. These works are numbered and the references to them throughout the manual will be by the author's name and the number.

An appendix with suggestions to instructors follows the bibliography. This contains matter which it is not necessary for the student to read in connection with his laboratory work, although in many cases it may be of interest for him to do so. The appendix is really a collection of practical notes based on the experience of the authors or gathered from conversations and correspondence with many teachers. Indeed, it is a feature which the authors have introduced in the hope that it may bring forth other helpful and practical suggestions from those who use the book, and correspondence upon this subject is cordially invited.

A glossary gives a selected list of botanical terms, including the most important of those used in this manual and in the authors' *Principles of Botany.*

Only a few necessary abbreviations have been used, to economize space. As stated above, books listed in the bibliography are referred to by the author's name and number in the list. *Principles* designates the *Principles of Botany;* App., the appendix; l.p., m.p., and h.p. refer to low power, medium power, and high power of the compound microscope respectively; lens means either hand lens or dissecting microscope as the case may be; c.p. means chemically pure. The usual abbreviations for the units of the metric system are frequently employed.

LABORATORY METHODS AND EQUIPMENT

The Laboratory and its Equipment

The essentials of a laboratory are, of course, good light, convenient tables, and sufficient apparatus. While north light is preferable, since its quality is more constant, east, west, or south light can be perfectly regulated by translucent shades which may be pulled up to any desired distance, and so temper direct sunlight when necessary. Moreover, it is desirable that some windows have the sun for part of the day, since aquaria and glass cases for growing plants require some sunlight and may be placed in such parts of the room. Excellent suggestions on the arrangement of laboratory tables, lockers, glass growing case, sink, blackboard, etc., are given in *Ganong*, 7, Chapter V, and in *Lloyd*, 8, Chapter IX, books which should be read by every teacher of botany.

The equipment of a laboratory will depend largely upon the nature of the work, whether very elementary or covering a strong full course of a year or more, and also upon the attitude of the instructor, who may emphasize especially either physiology or a more detailed morphology. Physiology requires its own special apparatus, and detailed morphology demands the equipment necessary for imbedding, microtome section cutting, and staining. Much of the work with this apparatus can best be conducted at tables in the center or back of the laboratory, which will not interfere with the tables for the more general class exercises. In the choice of equipment and its storage the instructor is again referred to the admirable discussions of *Ganong* and *Lloyd*. Lists of the chemicals, apparatus, and supplies necessary for the work outlined in this manual are given in Secs. 215, 216.

The cost of compound microscopes is the item of greatest expense in the equipment of a laboratory, and their selection

demands careful thought. The laboratory should have enough microscopes so that every student in a section may have his own instrument. If this is not possible, it is better that the course should be planned along such lines that the microscopic work is largely in the nature of demonstrations by the instructor on such microscopes as are available. Two or three students working together at the same microscope create confusion and secure poor results. There are a number of medium-priced instruments on the market, with varying merits, from which the instructor must choose for himself. A list of the more prominent firms and agents is given in Sec. 218. It is false economy to attempt to save expense on microscopes at the cost of workmanship and convenience in form. A set of microscopes may readily be kept on the laboratory tables, protected from the dust when not in use by paper cones, and used by successive sections, although this system demands much more watchfulness on the part of the instructor than when each student has his own instrument and is held responsible for its care.

Growing Plants in the Laboratory

Window sills and unused space should be utilized as far as possible for keeping fresh and growing material alive in the laboratory, not only for the interest that it arouses but also as a practical matter of foresight which at times saves much difficulty. Large jars covered with plate glass make excellent aquaria and give little or no trouble. A surprising number of forms will appear in them from time to time, and very interesting cultures frequently become established. A glass growing case (Wardian case) such as is described by *Ganong*, 7, p. 82, is a most useful piece of equipment, and practically indispensable for much physiological work when conservatories or greenhouses are not available. A bay window shut off from the rest of the room by tight glass screens is better still if the heat can be regulated.

Laboratory Material, Preparations, and Collections

A laboratory should be kept well stocked with material and slides sufficient for its work so that the instructor is never at a loss for them. Some material and slides will probably have to be purchased, and a list of dealers in botanical supplies is given in Sec. 217. However, very many instructors will depend chiefly on their own preparations and collections, and it is very desirable that they do so. Material collected and prepared by oneself will be generally better known and better taught than that from dealers. The secret of keeping a laboratory well stocked is the foresight which never loses the opportunity to preserve a fortunate collection. The simpler methods of killing and preserving material are given in Sec. 172. There are no great difficulties of technique, and it is the experience of every botanist that material will come to hand from time to time that is far better than the average of that offered by the dealers. A laboratory should always have large bottles of stock solutions of the simpler killing reagents (such as chrom-acetic acid) and preserving fluids (such as alcohol) and a supply of wide-mouthed bottles and jars. With this simple equipment at hand the instructor should be constantly on the watch for opportunities to increase and improve the laboratory stock. Thoughtfulness in this direction will save much time and expense in the long run.

It is becoming desirable and even necessary to study many points of detailed morphology and cell structure from slides. These can be purchased singly or in sets from dealers (Sec. 217) and the preparations are generally good; however, the instructor is urged to be self-reliant. The simpler methods of killing, imbedding, cutting, and staining are not difficult and are outlined in the sections entitled Botanical Microtechnique. An advanced student under direction can profitably be employed from time to time in the service of slide making with excellent returns for the expenditure involved. But more important is the added value

of working with material that is thoroughly familiar. There is danger in depending too much on slides, and they should not be used where the student may readily make temporary preparations, for much of the value of laboratory work lies in the development in the student of a certain manual skill. It is, however, still more important that he become acquainted with and study material first-hand. Botany made too easy by doing for the student what he can do for himself is botany robbed of certain of its most obvious advantages as a laboratory study.

Some instructors are making considerable use of the lantern and photographs, especially to illustrate ecological subjects, and for this purpose they are of the greatest service. Large and varied selections of lantern slides may be purchased (Sec. 219). Charts have their evident value and there are some excellent, although expensive, sets published (Sec. 219). It is not difficult to make simple charts and diagrams even in colors (*Ganong*, 7, p. 115), and these may be adapted to the particular needs of the course and cost almost nothing.

The herbarium and museum are most useful adjuncts to the laboratory. Especially important is demonstration material of groups which cannot be studied in many regions from living plants, as, for example, the marine algæ. Such material, either in the form of herbarium sheets or on exhibition in museum cases, forms a most useful part of the equipment of a botanical department. The advantages of collections covering the local flora are too obvious to need discussion. These matters are well treated by *Ganong*, 7, Chapter VI.

Laboratory Methods

The laboratory work, with its accompanying notes, should be kept absolutely separate from the text reading. Text-books should not be allowed on the laboratory tables. Their function is to present systematized accounts and conclusions after the student has obtained a sufficient first-hand knowledge of the

facts from the plants themselves, and to weld into one systematic whole the somewhat isolated topics of laboratory study. It is essential to good laboratory methods that the drawing and writing of notes be done in the laboratory, which should be regarded as a study room, like a library, open to the student as many hours of the day as is possible, and every encouragement should be given to extended individual work.

The Laboratory Equipment of Each Student

Every student should have an individual equipment, kept either in the drawers of the table or in lockers at the side of the laboratory. The following essential instruments and supplies had best be purchased by himself.

1. A razor, scalpel, forceps, and pair of needles.
2. Slides and cover glasses.
3. Four solid watch glasses or salt dishes.
4. Two pipettes (medicine droppers), a camel's-hair brush, and a scale in centimeters, millimeters, and inches.
5. A medium pencil (4H) or two pencils, hard (6H) and rather soft (3H), eraser, mapping pens, liquid India ink, red ink, and blue ink. Several colored pencils will be found very useful if the student is to construct diagrams illustrating life histories and other topics (App., 18). Higgins' red-label India ink runs more smoothly and is generally more satisfactory than the waterproof ink.
6. Drawing paper and notebook. The drawings required may be made on loose sheets kept in a folder, and the notes in a book, but it has generally proved more convenient to use perforated sheets of both drawing paper and note paper, cut to the same size, which can be loosely held together between stiff covers by a string. Such paper can be purchased in blocks from certain dealers (Sec. 218), or may be made up by a local stationer. The drawing paper should take ink as well as fine pencil lines.
7. A hand lens is necessary unless the laboratory tables are supplied with simple dissecting microscopes.

There should always be a general supply of glass tumblers, plates, saucers, etc., to hold material, and a set of the simpler reagents, such as iodine, eosin, acetic acid, potash solution, glycerin, etc. (Secs. 169, 170) may be placed on each table.

General Directions for the Student in Drawing and Recording Notes

1. Plan your drawings so that every sheet covers a definite subject or part of a subject and is not a mixture of unrelated matter. There are three types of drawings, *habit sketches*, *diagrams*, and *detailed figures*, which should never be combined in the same outline. The habit sketch and diagrams treat of general features, usually on a scale which makes it impossible to show details, which, if included, would either be out of proportion and inaccurate, or on too small a scale to be of value. Treat the drawings as a form of expression which should have the characteristics of good English, — namely, simplicity, clearness, and accuracy.

2. Depend chiefly on accurate outlines. Shade as little as possible, and then simply and effectively (see *Principles*, Figs. 8, 20, 113, 134, 168, 247, 273, 299). Do not put in details which you imagine but cannot see. Do not make objects appear more geometrically regular than they really are; peas are not perfectly spherical, pith cells seen in section never have the outlines of perfect hexagons, and so on.

3. Group your figures in an orderly manner, so that they tell a consecutive story on the page, as illustrated in the *Principles* by Figs. 8, 212, 270, and 299.

4. Ink drawings are more durable than pencil, but the manipulation requires a sure touch and some delicacy of treatment. They are best preceded by light pencil outlines, to establish proportions, which may be erased when the figure is finished. Use an India ink, diluted if necessary with weak ammonia water so that it will flow smoothly. Ink drawings are worth

trying and are generally favored by those with aptitude for illustration.

5. Describe the figures either neatly in a legend at the bottom of the sheet or on an accompanying page of the notes, using letters to refer to the parts indicated. For sample legends see *Principles*, Figs. 3, 57, 58, 59, 169, and 248. Give the approximate magnification when this is not evident. Explain in the notes all the points not shown in the sketches, such as characteristic color, consistency, etc. Think out everything before beginning to write a description, and, if it is lengthy, draw up a brief outline so that your notes have an orderly arrangement like the form of an essay. Write the notes in connection with the material and in the laboratory.

6. In describing an experiment record in separate paragraphs what you did, what the results were, and your conclusions from them. Do not leave out any little detail that may have influenced the results; for instance, if in a germination experiment the seeds were allowed to get too dry. Make your record on the spot. Do not go to the laboratory to observe the progress of an experiment and write part or all of your notes elsewhere, but put down the results in the presence of the materials and apparatus used.

7. If not original with yourself, always record the source from which any statements were obtained, thus: "Experiment IX, Results obtained by instructor in performing the experiment before the class."

8. Be neat and accurate. Forty pages of well-written, clearly expressed, and exact notes are worth more than a hundred pages of disorderly and inaccurate ones.

The Construction and Use of the Compound Microscope

A. The chief parts of a compound microscope are:

1. The *base* which rests on the table, generally horseshoe in form.

2. The *stage*, a horizontal shelf upon which is placed the preparation or *slide* to be examined. The stage is attached to the *column*.
3. The *mirror*, situated below the stage, by which the light is reflected upward through the opening in the stage.
4. The *diaphragm* of various forms, frequently accompanied by light *condensers*, attached to the lower side of the stage and used to regulate the intensity of the light reflected by the mirror.
5. The *tube*, a cylinder which holds the *lenses* and moves up and down perpendicularly above the opening in the stage. The tube is raised or lowered either by sliding it back and forth with a turning movement or by a rack and pinion mechanism. This mechanism is called the *coarse adjustment*.
6. The *fine adjustment*, a milled head back of the tube, which, on being turned, moves for a very short distance the entire framework that holds the tube.
7. The *lenses*, of two sorts, — *eyepieces* or *oculars* which slip into the upper end of the tube, and *objectives* which screw in at the bottom. An important accessory to the tube is the *nose piece*, capable of carrying two or three objectives which may be revolved into place at the lower end of the tube. A student's microscope will generally be fitted with two eyepieces, high and low, and with two objectives, high and low, and these may be combined with one another to give four grades of magnification ranging generally from about 50 to more than 500 diameters. If the objectives are respectively $\frac{2}{3}$ inch and $\frac{1}{6}$ inch and the eyepieces 2 inches and 1 inch, the lower objective with either eyepiece will give a *low power*, the higher objective with the 2-inch eyepiece a *medium power*, and the higher objective and 1-inch eyepiece a *high power*.
8. The *stand* consisting of the microscope without the lenses.

B. To set the microscope up:

1. Lift it out of its case by the lower part of the column to which the stage is attached, never by the tube or where the fine adjustment operates.
2. Place it on the table with the fine adjustment nearest you.
3. Screw the objectives into the nose piece and slip an ocular into the upper end; turn the lowest power objective into position.
4. Find the light by looking into the eyepiece and at the same time turning the mirror at such an angle that it reflects light from the window up through the opening in the stage to the objective. When a clear, bright field is obtained the microscope is *set up.*
5. Regulate the quantity of the light by the diaphragm. If too bright it must be cut off somewhat. The higher powers require brighter light than the lower. Mirrors generally have two faces, a plane and a concave. The concave mirror is used with the high-power objectives.

C. To find the object:

1. Place the slide on the stage, which should always be horizontal, with the object over the middle of the opening through which light is thrown from the mirror.
2. With the lower power in position move the coarse adjustment until either the object or small solid particles on the slide appear distinctly, which means that the lenses are *in focus.* The object, if not under the lens, may now be brought into the field by moving the slide back and forth very slowly. The focus of the coarse adjustment may generally be improved upon by the fine adjustment.
3. To focus with the high-power objective, first find the object with the low power and arrange in the center of the field. Then turn the high-power objective into position. In well-made instruments it will generally be found to come nearly into focus, and a slight movement of the fine adjustment will show the object clearly. If not in focus, move

the tube slowly downward until the objective nearly touches the slide, watching it carefully from the side, and then raise it by the fine adjustment until the focus is established. Never *focus down* with the high-power objective because of the danger of pressing it into the slide and ruining the delicately mounted lenses.

D. Studying an object:

1. Always examine an object first with the low powers so as to understand its general structure before passing to details.
2. Obtain greater magnification, if the instrument permits, by using more powerful objectives rather than higher eyepieces, for owing to peculiarities of the lenses clearer images are thus obtained.
3. Do not rest satisfied until the light is of the best quality obtainable with the mirror and diaphragm. It should not be too bright. Details are shown more clearly by subdued light.
4. Keep both eyes open in using a microscope. If this is at first distracting, cover the free eye with the fingers or by a paper screen projecting from the microscope tube until it is no longer attracted by surrounding objects on the table and the attention is entirely concentrated on the working eye. Never let the habit of squinting develop.
5. If it is necessary to ascertain the exact size of an object this can best be done by the use of two *micrometers.* The *eyepiece micrometer* consists of a disk of glass ruled with fine equidistant lines; this is inserted beneath the upper lens of the eyepiece. The *stage micrometer* is a glass slide ruled with fine lines 1-100 mm. apart. To measure an object the number of spaces on the eyepiece micrometer which its image covers must be noted. Then the value of each space is to be ascertained by substituting for the object the stage micrometer. A simple calculation will now give the diameter of the object.

E. Rules for the use of the microscope:

1. Never allow the objective to touch the cover glass or the liquid in which the object is mounted.
2. Do not handle the front lens of the objective or unscrew the sections in which the lenses are mounted.
3. Clean the front lens of the objective only when necessary, and then with small pieces of lens paper, which should be thrown away after use, or with an old clean, soft handkerchief. Breathe on the lens before cleaning it, or, if that is not sufficient, moisten the lens paper with a drop of alcohol or xylol, taking care to wipe it perfectly dry as quickly as possible.
4. Do not let the objective remain long near volatile corrosive liquids such as hydrochloric or nitric acid or strong solutions of iodine.
5. Do not allow liquids to run from the slide over the stage or other parts of the microscope.
6. Keep the microscope covered with a bell jar or paper cone when not in use, and keep the objectives and eyepieces away from dust.

Part I

STRUCTURE AND PHYSIOLOGY OF SEED PLANTS

INTRODUCTORY STUDY OF A SEED PLANT AND ITS ORGANS

1. The common dwarf nasturtium (Tropæolum).[1]

A. *The plant body.* Take a plant which has been carefully dug up and note the division of the plant body into three sets of parts or organs, *roots*, *stems*, and *leaves*, which constitute its main bulk.

Make a reduced drawing to show the general form and proportions of the entire plant.

B. *Roots.* Note the general form and arrangement of the roots and the differences between roots and stem in size, shape, color, and texture.

C. *Stem.* Make a reduced drawing of a portion of the stem, with parts of two or three leafstalks, showing how they are attached to it. Does the stem branch? Is it solid or hollow?

D. *Leaves.* Make a reduced drawing of one of the largest leaves, including the *leafstalk*, and life-size drawings of two or three of the youngest leaves near the tip of the stem. Note the mode of attachment of the leafstalk to the expanded portion, *blade*, of the leaf, the course of the veins through the blade, and the differences between the upper and lower surfaces of the latter.

[1] Any plant with well-developed roots, stems, and leaves, and simple, conspicuous flowers, will answer for this study. Good types available in autumn are the garden balsam, the wild yellow oxalis (*O. cymosa*), the petunia, any of the *Gerardias*, etc.

Roots, stems, and leaves taken together constitute the *vegetative organs* of the plant body, or the apparatus by which it carries on the processes necessary for its life and growth. In a general way it may be said that the roots serve to anchor the plant and to absorb water and dissolved raw materials from the soil to aid in the manufacture of plant food, that the stem conducts water and plant foods, and that the leaves carry on most of the work of food making for the plant and of admitting oxygen for respiration.

E. *The flower.* Note the occurrence of flowers at intervals along the stem. Locate the points from which flowers may arise. Sketch a short section of the stem with a flower attached. Make a drawing of a flower (side view), noting the *spur* which extends for some distance nearly parallel to the flower stalk. Examine the outer surface and the inner surface of the flower to see how the somewhat leaf-like but bright-colored parts which inclose it are related to each other. The five outer portions together make up the *calyx*, and the five inner ones the *corolla*. Calyx and corolla together constitute the *perianth*. Cut away the members of the corolla and note in the interior of the flower the eight curved stalks, each surmounted by a knob, and within them a smaller object, split at the tip into three divisions. The knobbed organs are *stamens* and the innermost organ is a *pistil*.

F. *The fruit.* Find a series of old flowers in which the calyx and corolla have become more and more withered, and trace the development of the lower part of the pistil into a green three-lobed *fruit*. Cut across a large, nearly dry fruit and find out how many seeds are contained in each of its divisions.

2. Reproduction in the seed plant. Stamens and pistils taken together constitute the *reproductive organs* of the plant. The calyx and corolla aid the work of the stamens and pistils in various mechanical and other ways.[1] The use of the flower is to bear seed, and seed formation is brought about by the action

[1] See *Principles*, Chapter XXXII.

of the *pollen* (a substance produced by the stamens)[1] on the rudimentary seeds, known as *ovules*, borne within the divisions of the three-lobed base of the pistil.

3. Life history. The *life history* of every seed plant comprises the series of changes which it undergoes in springing from a seed, growing to maturity, and producing flowers and seed of its own.

THE SEED AND ITS GERMINATION

4. Germination of the squash seed.* * Soak some squash seeds in tepid water for twelve hours or more. Plant these about an inch deep in damp sand, pine sawdust, or peat moss, in a wooden box which has had holes enough bored through the bottom to prevent its holding water. Put the box in a warm place (not at any time over 70°–80° Fahrenheit, or 21°–27° Centigrade), and cover it loosely with a board or a pane of glass. Keep the sand or sawdust moist, but not wet, and the seeds will germinate. As soon as any of the seeds, on being dug up, are found to have burst open, sketch one in this condition, noting the manner in which the outer seed coat is split. Look for the *peg*, a kind of knob, or hook, at the base of the *hypocotyl*, and see what it has to do with the actions of the seedling. Continue to examine the seedling at intervals of two days, until at least eight stages in the growth of the plantlet have been noted.

Observe particularly how the sand is pushed aside by the rise of the young seedlings. Suggest some reason for the manner in which the sand is penetrated by the rising stem.

5. Examination of the squash seed.* * Make a sketch of the dry seed, natural size.

A. Note the scar at the pointed end of the seed where the latter was attached to its place of growth in the squash. Label this *hilum*.

B. Note the little hole near the *hilum;* it is the *micropyle*, seen most plainly in a soaked seed.

[1] In the nasturtium the pollen is a yellow, rather sticky powder.

C. Describe the color and texture of the outer coating of the seed. With a scalpel or a very sharp knife cut across near the middle a seed that has been soaked in water for twenty-four hours. Examine with the dissecting microscope and sketch the section thus treated.

D. Taking another soaked seed, chip away the white outer shell, called the *testa*, and observe the thin, greenish, inner skin with which the kernel of the seed is closely covered.

E. Strip this off and sketch the uncovered kernel or *embryo*. Note that at one end it tapers to a point. This pointed portion, known as the *hypocotyl*, after the seed sprouts will develop into the stem of the plantlet. Split the "halves" of the kernel, seed leaves or *cotyledons*, entirely apart from each other, and note where and to what extent they are connected.

F. Have ready some seeds which have been soaked for twenty-four hours and then left in a loosely covered jar on damp blotting paper at a temperature of 70° Fahrenheit (21° Centigrade) or over until they have begun to sprout. Split one of these seeds apart, separating the cotyledons, and observe, at the junction of these, two very slender pointed objects, the rudimentary leaves of the *plumule* or first bud.

6. Examination of the bean. Study the seed, both dry and after twelve hours' soaking, in the same way in which the squash seed has just been examined.

A. Notice the presence of a distinct plumule, consisting of a pair of rudimentary leaves with a minute stem, between the cotyledons, just where they are joined to the top of the hypocotyl. In many seeds (as the pea) the plumule does not show the distinct leaves, but in all cases it contains the *growing point*, the tip of the stem from which all the upward growth of the plant is to proceed.

B. Make a sketch of these leaves as they lie in place on one of the cotyledons, after the bean has been split open.

Note the cavity in each cotyledon caused by the pressure of the plumule and of the hypocotyl.

7. Examination of the pea. There are no very important points of difference between the bean and pea, so far as the structure of the seed is concerned, but the student should rapidly dissect a few soaked peas to gain an idea of the appearance of the parts, since he is to study the germination of the pea in detail.

Make only one sketch, that of the hypocotyl as seen in position after the removal of the seed coats.

8. Germination of the bean (or the white lupine), the pea, and the grain of corn.* Soak some beans or lupine seeds as directed in Sec. 4, plant them, and make a series of sketches on the same general plan as those in *Principles*, Fig. 8.

Follow the same directions with some peas and some corn. In the case of the corn, make six or more sketches at various stages to illustrate the growth of the plumule and the formation of roots. The student may be able to discover what becomes of the large outer part of the embryo. This is believed to be the single cotyledon of the corn. It does not as a whole rise above ground, but most of it remains in the buried grain, and acts as a digesting and absorbing organ through which the *endosperm*, or food stored outside of the embryo, is transferred into the growing plant as fast as it can be made liquid for that purpose.

9. Germination of the horse-chestnut. Plant some seeds of the horse-chestnut or the buckeye, study their mode of germination, and observe the nature and peculiar modification of the parts.

EXPERIMENT I*

Relation of temperature to germination.* Prepare at least four beakers or tumblers, each with wet, soft paper packed in the bottom to a depth of nearly an inch. Have a tightly fitting cover, such as a square of window glass or a "clock glass," over

* To the Instructor: As some of the experiments upon seeds occupy a good many days or weeks for their completion, the laboratory work should be pushed on without waiting until these are finished. Results may be discussed from time to time while the experiments are in progress and summed up when they are entirely finished.

each. Put in each vessel the same number of soaked peas of about the same size. Stand the vessels with their contents in places where they will be exposed to different, but fairly constant, temperatures, and the same conditions as regards light, and observe the several temperatures carefully with a thermometer. Take pains to keep the tumblers in the warm places from drying out, so that their contents will not be less moist than those of the others. The following series is merely suggested; other values may be found more convenient. Note the rate of germination in each place and record in tabular form as follows:

No. of seeds sprouted in	24 hr.	48 hr.	72 hr.	96 hr.	etc.
At 32° F. (0° C.)	——	——	——	——	
At 50° F. (10° C.)	——	——	——	——	
At 70° F. (21° C.)	——	——	——	——	
At 90° F. (32° C.)	——	——	——	——	

If a thermostat can be had, it should be used to control the temperatures, and the highest point at which germination can take place should be noted.

EXPERIMENT II

Amount of water in air-dry seeds and amount absorbed to produce germination.

A. Weigh accurately a convenient quantity of seeds, and then dry them on the water bath until they no longer lose weight.[1] Report the loss of weight as water and calculate what per cent it constituted of the total weight.

B. Weigh a new set of seeds from the original (undried) lot, place them between layers of porous white paper kept thoroughly moist but not dripping wet, cover them, and allow them to remain until the germination is evidently begun. Reweigh the seeds, and calculate the increase of weight by absorption of water and the per cent of absorbed water.[2] This last will be

$$\frac{\text{weight water absorbed}}{\text{weight air-dry seeds}}.$$

[1] This may be done once for all for the entire laboratory division.

[2] The gain in weight observed may be a trifle less than the total value, since some loss of weight by oxidation is certain to have occurred.

EXPERIMENT III

Will seeds germinate well without a good supply of air? * *

A. Place some soaked seeds on damp blotting paper in the bottom of a bottle, using seeds enough to fill it three quarters full, and close tightly with a rubber stopper.

B. Put a few other seeds of the same kind in a second bottle, and cover loosely. Place the bottles side by side, so that they will have the same conditions of light and heat. Watch for results and tabulate as in previous experiments.

EXPERIMENT IV

Effect of germinating seeds upon the surrounding air.** When Exp. III has been finished remove a little of the air from above the peas in the first bottle. This can easily be done with a rubber bulb attached to a short glass tube. Then bubble this air through some clear limewater made by slaking quicklime in warm water and filtering through a paper filter. Also blow the breath through some limewater by aid of a short glass tube. Explain any similarity in results obtained. (Carbon dioxide turns limewater milky.) Afterwards insert into the air above the peas in the same bottle a lighted pine splinter, and note the effect upon its flame.

STORAGE OF FOOD IN THE SEED

EXPERIMENT V

Are the cotyledons of a pea of any use to the seedling? Sprout several peas on blotting paper. When the plumules appear carefully cut away the cotyledons from some of the seeds. Place on a wide, perforated cork one or two seedlings from which the cotyledons have been cut, and as many which have not been mutilated. Put the cork in the mouth of a cylindrical glass jar of water, which it should fit moderately well, and allow the roots to extend into the water, which must be kept always at the same level. Let them grow for some weeks and note results.

EXPERIMENT VI

Does the amount of material in the seed have anything to do with the rate of growth of the seedling? Germinate ten or more clover seeds, and about the same number of peas, on moist blotting paper under a bell jar. After they are well sprouted transfer both kinds of seeds to fine cotton netting, stretched across wide-mouthed jars nearly full of water. Only the roots of the seedlings should touch the water. Allow the plants to grow until the peas are from four to six inches high.

10. Examination of the four-o'clock seed.[1] Examine the external surface of a seed of the four-o'clock,[2] and note the hardness of the outer coat. From seeds which have been soaked in water at least twenty-four hours peel off the coatings and sketch the kernel. Make a cross section of one of the entire soaked seeds and sketch the section as seen with the magnifying glass, to show the parts, especially the two cotyledons, lying in close contact and encircling the white, starchy-looking *endosperm*. With a mounted needle pick out the little almost spherical mass of endosperm from inside the cotyledons of a seed which has been deprived of its coats, and sketch the embryo, noting how it is curved so as to inclose the endosperm almost completely.

11. Examination of the kernel of Indian corn.** Soak some grains of large yellow field corn for about two days.

A. Sketch an unsoaked kernel so as to show the grooved side, where the germ lies. Observe how this groove has become partially filled up in the soaked kernels.

B. Remove the thin, tough skin from one of the latter and notice its transparency. This skin — the bran of unsifted corn meal — does not exactly correspond to the testa and inner coat of ordinary seeds, since the kernel of corn, like all other grains (and like the seed of the four-o'clock), represents not merely the seed but also the seed vessel in which it was formed and grew, and is therefore a fruit.

C. Cut sections of the soaked kernels, some transverse, some lengthwise and parallel to the flat surfaces, some lengthwise and at right angles to the flat surfaces. Try the effect of staining some of these sections with iodine solution. Make a sketch of one section of each of the three kinds, and label

[1] Strictly speaking a fruit.

[2] Morning-glory seeds or grains of buckwheat also answer well.

the dirty white portion, of cheesy consistency, *embryo;* and the yellow portions, and those which are white and floury, *endosperm.*

D. Chip off the endosperm from one kernel so as to remove the embryo free from other parts. Notice its form, somewhat triangular in outline, sometimes nearly the shape of a beechnut, and in other specimens nearly like an almond. Estimate what proportion of the entire bulk of the soaked kernel is embryo (*Principles,* Fig. 378). Split the embryo lengthwise so as to show the slender plumule.

12. Recognition of some chemical compounds found in plants.** Out of the very numerous substances which make up the framework of the plant body, or are stored in it, there are several most important ones which the student should be able to recognize by simple tests. In this place only starch, sugar, cellulose, lignin, oil, and proteids will be discussed.

A. **Starch.** This turns blue, or nearly black, on the addition of iodine solution. Make the test on a bit of laundry starch the size of a grain of wheat diffused in a large test tube full of boiling water; it does not form a true solution. Add the iodine solution (Sec. 169) drop by drop to the boiled starch after the latter has cooled.

B. **Sugar.** Some of the sugars found in plants produce a yellow or orange color or an orange precipitate on being heated to boiling with a solution of copper known as Fehling's solution (Sec. 170). Cane sugar does not give the reaction readily unless it has first been boiled with dilute hydrochloric acid, when it responds promptly to the test. Make the test with Fehling's solution on a rather dilute solution of commercial glucose in hot water.

C. **Cellulose.** This turns blue on being moistened with iodine solution and then with concentrated sulphuric acid diluted with half its bulk of water. Make the test with a bit of absorbent cotton (in this particular case wetting the cotton first with the acid and then with the iodine solution).

D. **Lignin.** This substance, which forms a large part of the material of lignified cell walls, gives a reddish violet color with phloroglucin solution (Sec. 170) after the addition of hydrochloric acid. Make the test by moistening a thin shaving of any kind of light-colored wood with a solution of as much phloroglucin as can be taken up on the point of a penknife in thirty or forty drops of 95 per cent alcohol. Then wet the moistened shaving with a little concentrated hydrochloric acid.

E. **Oil.** Oils may be recognized by their characteristic appearance as seen in minute droplets in the tissues of the plant when examined with the microscope. Thin sections containing oil, when treated with ether or chloroform, lose the oil almost instantly. Oils (and resins also) are colored a deep red by the alcoholic solution of alkannin (Sec. 170) or of the soluble material in alkanet root. Make the test on a thin section of an oily seed (not *Ricinus* seed) placed under the microscope in an alcoholic solution of alkannin.

F. **Proteids.** Proteids usually give a brick-red or rose-red color when moistened with Millon's reagent (Sec. 170) and gently heated. They are stained yellow or brown by iodine solution. All proteids turn yellow (*xanthoproteic reaction*) when moistened with strong nitric acid and slightly warmed. The color deepens on the addition of ammonia water to the stained substance. To make the nitric-acid test, warm a little egg albumen with the strong acid, and when the coagulated albumen becomes decidedly yellow pour off the excess of acid and cover the stained mass with a little ammonia water.

REFERENCES. For the substances to be tested, *Principles;* Pfeffer, 31 ; for the tests themselves, Zimmerman's *Botanical Microtechnique* (Henry Holt & Co., New York), and Strasburger-Hillhouse, 6.

EXPERIMENT VII

Occurrence of starch in seeds. Cut in two with a sharp knife the seeds to be experimented on, and then pour on each, drop by drop, some iodine solution. Only a little is necessary ; sometimes the first drop is enough.

If starch is present a blue color (sometimes almost black) will appear. If no color is obtained in this way, boil the pulverized seeds for a moment in a few drops of water and try again.

Test in this manner corn, wheat (in the shape of flour), oats (in oatmeal), barley, rice, buckwheat, flax, rye, sunflower, four-o'clock, morning-glory, mustard seed (not ground mustard), beans, peanuts, Brazil nuts, hazelnuts, and any other seeds that you can get. Report results in tabular form.

REFERENCE. Strasburger-Hillhouse, 6.

13. Absorption of starch from the cotyledons. Examine with the microscope, using m.p. (medium power), thin sections of soaked beans and the

cotyledons from seedlings that have been growing for three or four weeks. Stain the sections with iodine solution, and notice how completely the clusters of starch grains that filled most of the cells of the unsprouted cotyledons have disappeared from the shriveled cotyledons of the seedlings.

REFERENCES. Strasburger-Hillhouse, 6; Tschirch, 74.

14. Structure of starch.* *

A. Cut moderately thin sections of a potato tuber, mount in water, and examine with m.p. (medium power). Note the *starch grains* inclosed in little chambers or *cells*. Run in a little weak iodine solution (Sec. 169) under one edge of the cover glass, at the same time withdrawing water from the opposite edge with a bit of blotting paper. Watch the section carefully during the process and note the gradual staining of the starch grains. Draw.

B. Mount in water some pulp scraped from a freshly cut surface of potato and examine with h.p. (high power). Move the fine adjustment constantly while observing, and note the lines arranged somewhat concentrically about a point called the *hilum*, often marked by minute cracks in the grain. Draw several grains.

Is there any evidence that the starch grain is composed of successive layers? If so, how may they have been caused?

C. Draw to the same scale as seen under h.p. all the principal forms and sizes of potato starch grains that you can find, together with grains of several other kinds, as canna starch (from the rootstock), oat starch, corn starch, and *Euphorbia* starch (from *E. splendens*).

REFERENCES. Strasburger-Hillhouse, 6; Tschirch, 74.

EXPERIMENT VIII

Determination of oil in flaxseed. Weigh out two ounces (or sixty grams) of ground flaxseed and add an equal volume of ether or benzine. *Do not bring these liquids near a gas jet or any other flame.* Let it stand ten or fifteen minutes and then filter. Wash the meal by pouring over it, a little at a time, about the same

amount of liquid as was used at first. Let the liquid stand in a saucer or evaporating dish in a good draft till it has lost the odor of the ether or benzine. Weigh the remaining oil and calculate what per cent of the ground seed was oil. (Traces will of course still be left in the residue on the filter.)

Describe the oil which you have obtained. Of what use would it have been to the plant?

EXPERIMENT IX

Detection of proteids in seeds. Extract the germs from some soaked kernels of corn and bruise them, or soak some wheat-germ meal for a few hours in warm water, or in a stream of water wash the starch out of wheat-flour dough; reserving the residue for use, place it in a white saucer or porcelain evaporating dish and moisten well and heat with Millon's reagent (Sec. 170) or with nitric acid; examine after fifteen minutes. Proteids turn yellow when moistened with nitric acid and red with Millon's reagent.

Reference. Strasburger-Hillhouse, 6.

EXPERIMENT X

What plant foods are found in Brazil nuts? Crack several Brazil nuts, peel off the brown coating from the kernel of each, and then grind the kernels to a pulp in a mortar. Shake up this pulp with ether, pour upon a filter paper, and wash with ether until the washings when evaporated are nearly free from oil. The funnel containing the filter should be kept covered as much as possible until the washing is finished. Evaporate the filtrate to procure the oil. Dry the powder which remains on the filter and keep it in a wide-mouthed bottle. Test some of it for starch and for proteids. Does it appear that a seed needs to contain both starch and oil, or may one replace the other?

15. Microscopical study of reserve oil in a seed. Cut moderately thin sections of an oily seed, e.g. peanut (not roasted). Mount in water and examine with m.p. Note the cellular structure of the seed and the minute oil globules within the cells. Try to estimate the number in a cell. Mount another section in an alcoholic solution of alkannin or of the soluble portion of alkanet root (Sec. 170). After a few minutes examine the section and note

the stained oil globules. Some larger droplets of oil may appear outside of the section. Sketch, using h.p. if necessary.

REFERENCES. Strasburger-Hillhouse, 6; Tschirch, 74.

16. Structure of proteid grains (aleurone grains). A large part of the proteid reserve material of seeds is stored in the form of minute bodies known as *aleurone grains.* They occur in abundance packed around the starch grains in such seeds as those of the bean and pea, but are more easily studied in seeds nearly or quite free from starch. Remove the testa from a seed of the castor-oil plant (*Ricinus*) and cut thin sections from the endosperm. Mount in olive oil (which does not dissolve any of the proteid material) and examine with h.p. Note the very small aleurone grains, each with a clear body at the narrow end. This clear body, called the *globoid*, is of mineral material, principally a double phosphate of lime and magnesia. Draw the aleurone grains. Mount another section in water and examine; then run in absolute alcohol under one edge of the cover glass, and note the *proteid crystal*, which should appear plainly, constituting a large part of the bulk of the aleurone grain, and the globoid. The latter is now distinctly recognizable as a solid substance. Draw.

Aleurone grains may be more easily demonstrated in thin sections of the kernel of the Brazil nut. These should be rinsed twice in chloroform, to remove the oil, then once in alcohol, and mounted in alcohol. Examine with h.p., run in iodine solution while under the microscope, and note the brown-stained grains in the cells. Draw.

REFERENCES. Strasburger-Hillhouse, 6; Tschirch, 74.

MOVEMENTS, DEVELOPMENT, AND MORPHOLOGY OF THE SEEDLING

EXPERIMENT XI

Is the arch of the hypocotyl due to the pressure of the soil on the rising cotyledons? Sprout some squash seeds on wet paper under a bell glass, and when the root is an inch or more long hang several of the seedlings, roots down, in little stirrups made of soft twine, attached by a mixture of equal parts of beeswax and rosin melted together to the inside of the upper part of the bell glass. Put the bell glass on a large plate or sheet of glass on which lies wet paper to keep the air moist. Note whether or not the seedlings form hypocotyl arches, and, if so, whether the arch is more or less perfect than that formed by seedlings growing in earth, sand, or sawdust.

EXPERIMENT XII

The permanganate test, to distinguish root from hypocotyl. Make a solution of potassium permanganate in water by adding about 4 parts, by weight, of the crystallized permanganate to 100 parts of water. Drop into the solution seedlings of all the kinds that have been so far studied, each in its earliest stage of germination (that is, when the root, or hypocotyl, has pushed out of the seed half an inch or less), and also at one or two subsequent stages. After the seedlings have been in the solution from three to five minutes, or as soon as the roots are considerably stained, pour off (and save) the solution and rinse the plants with plenty of clear water. Sketch one specimen of each kind, coloring the brown-stained part, which is root, in some way so as to distinguish it from the unstained hypocotyl. Note particularly how much difference there is in the amount of lengthening in the several kinds of hypocotyl examined. Decide whether the peg of the squash seedling is an outgrowth of the hypocotyl or of the root.

EXPERIMENT XIII

In what portions of the root does its increase in length take place? ** Sprout some peas on moist blotting paper in a loosely covered tumbler. When the roots are one and a half inches or more long, mark them along the whole length with equidistant dots made with a bristle dipped in waterproof India ink, or a fine inked thread stretched on a little bow of whalebone or brass wire.

Fasten the peas with pins to moist blotting paper placed in a vertical position under a bell glass or an inverted battery jar, and examine the roots at the end of twenty-four hours to see along what portions their length has increased; continue observations on them for several days.

REFERENCES. Detmer-Moor, 9; Pfeffer-Ewart, 31, II; Darwin and Acton, 11.

17. Review sketches. Make out a comparison of the early life histories of all the other seedlings studied, by arranging in parallel columns a series of drawings of each, like those of *Principles*, Fig. 8, but in vertical series, the youngest of each at the top, thus:

	Bean	Pea	Corn
First stage			
Second stage			
Third stage			
Fourth stage			
Fifth stage			

Discuss their resemblances and differences.

ROOTS

18. Growth and microscopical examination of water roots. * *

A. Place some vigorous cuttings of *Tradescantia*, which can usually be obtained of a gardener or florist, in a beaker or

jar of water. The jar should be as thin and transparent as possible, and it is well to get a flat-sided rather than a cylindrical one. Leave the jar of cuttings in a sunny, warm place.

B. As soon as roots have developed at the nodes, and reached the length of three quarters of an inch or more, arrange a microscope in a horizontal position (Fig. 1) and examine the

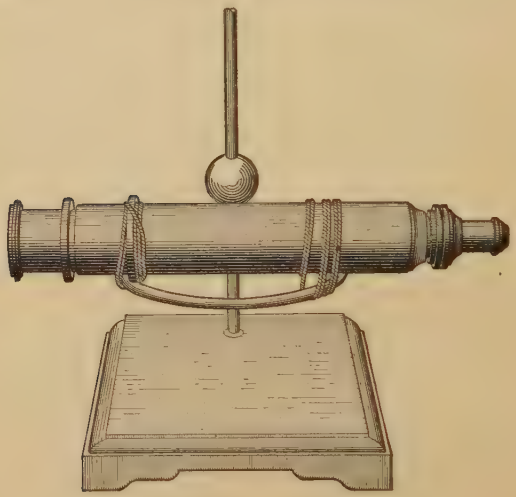

FIG. 1. Microscope on ring stand

tip and adjacent portion of one of the young roots with a power of from twelve to twenty diameters. Note:

(1) The root cap, of loosely attached cells.
(2) The central cylinder.
(3) The cortical portion, a tubular part inclosing the solid central cylinder.
(4) The root hairs, which cover some parts of the outer layer of the cortical portion very thickly. Observe particularly how far toward the tip of the root the root hairs extend, and where the youngest ones are found.

Make a drawing to illustrate all the points above suggested (1–4). Make a careful study of longitudinal sections through the centers of the tips of very young roots of the hyacinth or the "Chinese sacred lily."[1] Sketch one section.

Make a study of the roots of any of the common duckweeds, growing in nutrient solution (No. 1, Exp. XV) in a jar of water under a bell glass, and note the curious root pockets, which here take the place of root caps.

REFERENCES. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenk, Karsten, 1.

19. Structure of the central cylinder of a monocotyledonous root.** Cut thin cross sections of the adventitious roots of onion or hyacinth near their bases.[2] Examine these in water with a power of two hundred or more diameters.

The *central cylinder* or *stele* shows in the cross section as a nearly circular area containing a few large openings and many smaller ones. The largest openings are usually only one or two in number and represent the large *vessels* cut across. These are tubes with a diameter of $\frac{1}{300}$ to $\frac{1}{400}$ of an inch, with ladder-like markings (seen only in the longitudinal section) on their walls. Radiating away from these are the openings of (in the onion) six other vessels of about half the diameter of the central vessels and with similar markings. Just outside of each of the six vessels is an irregular group of much smaller vessels with spiral markings (seen on longitudinal section). The openings of the vessels form on the cross section of the central cylinder an irregular six-rayed star, and the spaces between the rays are mainly filled by *sieve tubes* or *soft bast*, separated from the vessels of the *wood* system by parenchyma cells. The outermost portion of the central cylinder consists of a single layer of cells constituting the *pericycle*, and this is surrounded by the innermost layer of the primary cortex, the *endodermis*.

REFERENCE. Strasburger-Hillhouse, 6.

20. Structure of the dicotyledonous root; secondary thickening.[3] The structure of very young dicotyledonous roots is often similar in most respects to that of the onion root (Sec. 19).[4] *Secondary thickening* (*Principles*, Sec. 80)

[1] *Narcissus Tazetta*, var. *orientalis*.

[2] These roots may be obtained from an onion or a hyacinth bulb set in a tumblerful of water and left in a warm place until the roots are well developed. They may also be taken from hyacinth plants growing in pots, by inverting the latter, removing the contents, and replacing the plant when the needed material has been secured from it.

[3] This section may to advantage be deferred until after Sec. 31.

[4] Good materials for study are roots of *Ranunculus*, bean, or (very young) grapevines.

soon occurs in the roots of dicotyledonous trees and shrubs, and the structure of such roots considerably resembles that of the stem, except that pith is frequently lacking.

With the lens examine cross sections of large roots of any hardwood tree. Note the *annual rings* of wood and their porosity, due to the presence of many and large *vessels*. The cortical part sometimes (as in sassafras) forms a thick bark.

With the microscope examine thin cross sections, stained with phloroglucin (Sec. 12, D), of the tap root of a seedling hardwood tree not more than a year old.[1] Use first l.p., then m.p. Note the division of the root into a cortical region or bark, wood, and (sometimes) pith. Note the relatively small amount of wood in the younger portions of the root, increasing in the older parts. Make drawings to illustrate this point. Make a drawing of a quarter or less of one of the older sections, showing the distribution of material from center to exterior. In your drawing color the lignified *hard-bast fibers* of the bark (Sec. 29, C) and the wood fibers, to distinguish them from the non-fibrous *parenchyma* which makes up much of the bulk of the young root.

If the material was collected in the autumn or winter, test a section with iodine solution for starch, and if any is found describe its distribution.

References. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenck, Karsten, 1; Tschirch, 83.

21. Examination of a fleshy root. Cut a parsnip across below the middle, and stand the cut end of the upper part in eosin solution (Sec. 169) for twenty-four hours.

A. Examine by slicing off successive portions from the upper end. Sketch some of the sections thus made. Cut one parsnip lengthwise and sketch the section obtained. In what portion of the root did the colored liquid rise most readily? The ring of red marks the exterior of the central cylinder in contact with the cortical portion. To which does the main bulk of the parsnip belong?

B. Cut thin transverse sections from an eosin-stained parsnip and notice how the medullary rays run out into the cortical portion, and in those sections that show it find out where the secondary roots arise.

[1] These may be planted for the purpose, but usually it is easy to find plenty of young seedling cherries, birches, elms, ashes, maples, etc.

C. If possible, peel off the cortical portion from one stained root and leave the central cylinder with the secondary roots attached. Stain one section with iodine and sketch it. Where is the starch of this root mainly stored?

D. Test some bits of parsnip for proteids by boiling them for a minute or two with strong nitric acid.

What kind of plant food does the taste of cooked parsnips indicate? [*On no account taste the bits which have been boiled in the poisonous nitric acid.*]

EXPERIMENT XIV

Percentage of water in the plant body. Take any such soft portions of seed plants as the roots of carrots or turnips, shoots of asparagus, and leaves of lettuce or spinach, or cut off a green herbaceous plant at the level of the ground. Slice the roots and stems as thin as possible and pick the leaves to pieces. Weigh out convenient portions at once to avoid drying, place each portion in a water bath, and heat until no further loss of weight takes place. It will save much time and render the experiment more accurate if the materials are in each case kept in a shallow vessel, such as a large watch glass, throughout the process of drying and the weighings. Finally calculate from the loss of weight the percentage of water originally present.

EXPERIMENT XV

What mineral substances are required by ordinary seed plants? * *

A. Prepare a nutrient solution (No. 1) containing for every 1500 parts by weight (grams) of water[1] the following amounts of salts:

	Grams
Calcium nitrate	2
Potassium chloride	$\frac{1}{2}$
Magnesium sulphate	$\frac{1}{2}$
Acid potassium phosphate (KH_2PO_4)	$\frac{1}{2}$
Ferric chloride solution	a few drops

Prepare several glass cylinders of the capacity of a pint or more by rinsing out with strong nitric acid and then with plenty of water.

[1] Distilled water which has been prepared in a glass, porcelain, or block tin distilling apparatus and then aërated by shaking up with air should be used. Very pure rain water collected from a thoroughly washed roof will answer equally well.

B. Make another nutrient solution (No. 2) like No. 1, but without iron; another (No. 3) containing the same ingredients as No. 1, except the calcium nitrate, for which one gram of calcium sulphate is to be substituted; and another (No. 4) like No. 1, except that acid sodium phosphate is to be substituted for the acid potassium phosphate.

C. Place in each jar a vigorous young wheat seedling with only its roots submerged, or a cutting of *Tradescantia*. Cover each jar with a piece of pasteboard wrapped around the glass so as to exclude light from the solution and put all the jars in a warm place but not in full sunlight. Change the nutrient solution every week and continue the culture for four or five weeks. If the roots seem dirty and slimy, allow the plants to stand for a day or two at a time with the roots in distilled water or a weak solution of calcium sulphate.

D. At the end of the period sketch all the plants and label as follows:

1. Culture in full nutrient solution.
2. Culture without iron.
3. Culture without nitrogen.
4. Culture without potassium.

What conclusions can you draw from the experiment?

References. Detmer-Moor, 9; Pfeffer-Ewart, 31, I; Peirce, 32.

EXPERIMENT XVI

Effect of diminished temperature on absorption of water by roots.

A. Transplant a tobacco seedling about four inches high into rich earth contained in a narrow, tall beaker or very large test tube (not less than $1\frac{1}{4}$ inch in diameter and six inches high).

B. When the plant has begun to grow again freely in a warm, sunny room, insert a chemical thermometer into the earth; this can best be done by making a hole with a sharp, round stick, pushed nearly to the bottom of the tube, and then putting the thermometer in the place of the stick. Water the plant well, and then set the tube in a jar of pounded ice which reaches nearly to the top of the tube. Note the temperature of the earth just before placing it in the ice. Cover the ice with cotton batting or a piece of flannel so that the stem and leaves of the plant will not be chilled by the proximity of the ice.

C. Observe whether the leaves of the seedling wilt, and if so, at what temperature the wilting begins.

D. Finally, remove the tube from the ice and place it in warm water (about 80° F. or 27° C.). Observe the effect and note the temperature at which the plant, if wilted, begins to revive.

E. Find an average between the wilting temperature and the reviving temperature. For what does this average stand? Repeat the experiment with oat seedlings.

REFERENCE. Pfeffer-Ewart, 31, I.

EXPERIMENT XVII

Do all parts of the root of the Windsor bean seedling bend downward alike? Fasten some sprouting Windsor beans with roots about an inch in length to the edges of a thick disk of pine wood or other soft wood in a soup plate partly full of water and cover them with a low bell jar.

Steel pins run through the cotyledons, as in Fig. 2, will hold the beans in place. Mark the roots, as in Exp. XIII, to see in what region the bending occurs; that is, whether in the older part or by the addition of new material at the tip. When the roots have begun to point downward strongly, turn most of the beans upside down and pin them in the reversed position. If you choose, after a few days reverse them again. Make sketches of the various forms that the roots assume and discuss these.

REFERENCES. Detmer-Moor, 9; Pfeffer-Ewart, 31, III.

EXPERIMENT XVIII

Does the Windsor bean root tip press downward with a force greater than its own weight? Arrange a sprouted bean as shown in Fig. 2,[1] selecting one that has a root about twice as long as the diameter of the bean and that has grown out horizontally, having been sprouted on a sheet of wet blotting paper. The bean is pinned to a cork that is fastened with beeswax and rosin mixture to the side of a little trough or pan of glass or glazed earthenware. The pan is filled half an inch or more with *perfectly clean* mercury, and on top of the mercury is a layer of water. The whole is closely covered by a large tumbler or a bell glass. Allow the apparatus to stand until the root has forced its way down into the mercury. Then run a slender

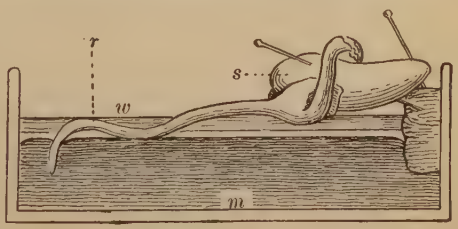

FIG. 2. A sprouting Windsor bean pushing its root tip into mercury

s, seed; *r*, root; *w*, layer of water; *m*, mercury
After Sachs

[1] Or see Ganong, 10.

needle into the root at the level of the mercury (to mark the exact level), withdraw the root, and measure the length of the part submerged in mercury. To see whether this part would have stayed under by virtue of its own weight, cut it off and lay it on the mercury. Push it under with a pair of steel forceps and then let go of it. What does it do?

EXPERIMENT XIX

What causes the root to go downward?

A. Pin some soaked Windsor beans to a large flat cork, cover them with thoroughly moistened chopped peat moss, and cover this with a thin glass crystallizing dish. Set the cork on edge.

B. Prepare another cork in the same way, attach it to a clinostat, and keep it slowly revolving in a vertical position for from three to five days. Compare the directions taken by the roots on the stationary and on the revolving cork.

REFERENCES. Ganong, 10; Pfeffer-Ewart, 31, III.

22. Propagation by means of roots. Bury a sweet potato or a dahlia root in damp sand and watch the development of sprouts from adventitious buds. One sweet potato will produce several crops of sprouts, and every sprout may be made to grow into a new plant. It is in this way that the crop is started wherever the sweet potato is grown for the market.

SOME PROPERTIES OF CELLS AND THEIR FUNCTIONS IN THE ROOT

EXPERIMENT XX

Osmosis as shown in an egg.

A. Cement to the smaller end of an egg a bit of glass tubing about six inches long and about three sixteenths of an inch in inside diameter. A mixture of equal parts of beeswax and rosin melted together makes the best cement for this. Chip away part of the shell from the larger end of the egg, place it in a wide-mouthed bottle or a small beaker full of water (as shown in *Principles*, Fig. 28), and then very cautiously pierce a hole through the upper end of the eggshell by pushing a knitting needle or wire down through the glass tube. Watch the

apparatus for some hours and note any change in the contents of the tube or the beaker.[1] Explain.

The rise of liquid in the tube is evidently due to water making its way through the thin membrane which lines the eggshell, although this membrane contains no pores visible even under the microscope.

B. An alternative experiment is to fasten a pig's bladder or a diffusion shell (obtainable of dealers in chemical and physical apparatus) to the end of a glass tube six or eight feet long. For a $\frac{5}{8}$-inch (16-mm.) diffusion shell the tube should be $\frac{5}{8}$-in. outside diameter; for the bladder a tube must be chosen that barely enters the opening in it. A tight joint is more certainly secured by using a tube a little smaller than is needed to enter the opening in the shell or bladder, slipping over the tube a bit of rubber tubing an inch or more long, inserting this in the shell and wiring it tightly with rather fine copper wire. Fasten the tube upright, with the diffusion membrane submerged in a large jar of water, and pour into the open end of the tube enough molasses to remain visible above the diffusion membrane. A rather large tube may be filled through a slender funnel, taking pains not to let the molasses stick to the sides as it descends. A narrow tube must be filled before tying into the neck of the bladder, the free end of the tube corked, and the other end then tied in place. Note any change of level in the molasses in the tube.[2]

REFERENCES. Ganong, 10; Detmer-Moor, 9; Pfeffer-Ewart, 31, I.

EXPERIMENT XXI

Result of placing sugar on a begonia leaf. Put a little powdered sugar on the upper surface of a thick begonia leaf under a small bell glass. Put another portion of sugar on a bit of paper alongside the leaf. Watch for several days. Explain the results. The *upper* surface of this leaf contains no pores, even of microscopic size.

STEMS

23. The horse-chestnut or buckeye twig.** Procure a twig of horse-chestnut eighteen inches or more in length. Make a careful sketch of it, trying to bring out the following points:

A. The general character of the bark.

[1] Testing the contents of the beaker with a solution of nitrate of silver will then show the presence of more common salt than is found in ordinary water.

[2] A still more instructive experiment is that on plasmolysis of the *Spirogyra* cell (Secs. 56, D, and 57, C).

B. The large horseshoe-shaped scars and the number and position of the dots on these scars. Compare a scar with the base of a leafstalk furnished for the purpose.

C. The ring of narrow scars around the stem in one or more places, and the different appearance of the bark above and below such a ring.[1] Compare these scars with those left after removing the scales of a terminal bud.

D. The buds at the upper margin of each leaf scar and the strong terminal bud at the end of the twig. The dots on the leaf scars mark the position of the ducts and wood cells in the fibro-vascular bundles which run from the wood of the branch through the leafstalk up into the leaf.

E. The flower-bud scar, a concave impression to be found in the angle produced by the forking of two twigs, which form, with the branch from which they spring, a Y-shaped figure.

F. The place of origin of the twigs on the branch (on a branch larger than the twig handed round for individual study); make a separate sketch of this.

The portion of a stem which originally bore any pair of leaves is a *node*, and the portions between the nodes are *internodes*.

Describe briefly in writing alongside the sketches any observed facts which the drawings do not show.

If your twig was a crooked, rough-barked, and slow-growing one, exchange it for a smooth, vigorous one, and note the differences. Or if you sketched a quickly grown shoot, exchange for one of the other kind.

Questions. 1. How many inches did your twig grow during the last summer? How many during the summer before? How do you know? How many years old is the whole twig given you?

2. How were the leaves arranged on the twig? How many leaves were there? Were they all of the same size?

[1] Maple, box elder, or lilac may be used, though they are not nearly as good. Instead of beech, as described in the next section, basswood, any kind of hickory, butternut, black walnut, poplar, or cottonwood will do. The rings are especially well shown by cherry, apple, pear, cottonwood, or aspen.

3. What has the mode of branching to do with the arrangement of the leaves? with the position of the flower-bud scars?

24. Twig of beech.

A. Sketch a vigorous young twig of beech (or of hickory, magnolia, or tulip tree) in its winter condition, noting particularly the respects in which it differs from the horse-chestnut. Describe in writing any facts not shown in the sketch. Notice that the buds are not opposite, nor is the next one above any given bud found directly above it, but part way round the stem from the position of the first one.

B. Ascertain, by studying several twigs and counting around, which bud is above the first and how many turns round the stem are made in passing from the first to the one directly above it.[1]

C. Observe with especial care the difference between the beech and the horse-chestnut in mode of branching, as shown in a large branch provided for the study of this feature.

STRUCTURE OF THE STEM

Stem of Monocotyledonous Plants

25. Gross structure of the corn stem.** Refer to the sketches of the corn seedling to recall the early history of the corn stem.

A. Study the external appearance of a piece of corn stem or bamboo two feet or more in length. Note the character of the outer surface. Sketch the whole piece and label the enlarged *nodes* and the nearly cylindrical *internodes*.

B. Cut across a corn stem and examine the cut surface with the lens. Make some sections as thin as they can be cut and examine with the lens (holding them up to the light) or with a dissecting microscope. Note the firm rind composed of the epidermis and the underlying tissue, the large

[1] This may be made clearer by winding a thread about the twig, making it touch the base of each bud.

mass of pith composing the main bulk of the stem, and the many little harder and more opaque spots, which are the cut-off ends of the woody threads known as *fibro-vascular bundles.*

C. Split a portion of the stem lengthwise into thin, translucent slices, and notice whether the bundles seem to run straight up and down its length; sketch the entire section ($\times$ 2). Every fibro-vascular bundle of the stem passes outward through some node in order to connect with some fibro-vascular bundle of a leaf. Knowing this fact, the student would expect to find the bundles bending out of a vertical position more at the nodes than elsewhere. Can this be seen in the stem examined? Observe the thickening at the nodes, and split one of these lengthwise to show the tissue within it.

D. Compare with the corn stem a piece of palmetto and a piece of cat brier (*Smilax rotundifolia*, *S. hispida*, etc.), and notice the similarity of structure. Compare also a piece of rattan and of bamboo.

Minute structure.

E. Stain a thin cross section[1] with phloroglucin (Sec. 12, D) and sketch with m.p. one of the larger bundles (some distance in from the rind). In your drawing color the stained portions, which represent the lignified sclerenchyma fibers. Look for stained rigid tissue (sclerenchyma) in the rind.

F. Cut several very thin longitudinal sections from a piece of stem not more than one-fourth to one-third inch long, split through the middle. Stain with phloroglucin and make a drawing of the best bundle found. Note the two kinds of vessels, or vessel-like tracheids, some with spiral threads lining the interior, and others with transverse rings. Separate rings are often seen detached from their vessels and beautifully stained by the phloroglucin.

REFERENCES. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenck, Karsten, 1.

A more complicated kind of monocotyledonous stem structure can be studied to advantage in the surgeons' splints cut from yucca stems and sold by dealers in surgical supplies.

[1] Asparagus stem may also be used.

Stem of Dicotyledonous Plants

26. Gross structure of an annual dicotyledonous stem.**

A. Study the external appearance of a piece of sunflower stem several inches long. If it shows distinct nodes, sketch it.

B. Examine the cross section with the lens and sketch it. *After your sketch is finished* compare it with *Principles*, Fig. 56, which probably shows more details than your drawing, and label the parts shown as they are labeled in that figure.

C. Split a short piece of the stem lengthwise through the center and study the split surface with the lens. Take a sharp knife or a scalpel and carefully slice and then scrape away the bark until you come to the outer surface of a bundle.

D. Examine a vegetable sponge (*Luffa*), sold by druggists, and notice that it is simply a network of fibro-vascular bundles. It is the skeleton of a tropical seed vessel or fruit, very much like that of the wild cucumber common in the central states, but a great deal larger.

Structure of bark. The different layers of the bark cannot all be well recognized in the examination of a single kind of stem. With lens examine:

E. The *cork* which constitutes the outer layers of the bark of cherry or birch branches two or more years old. Sketch the roundish or oval *lenticels* on the outer surface of the bark. How far in do they extend?

F. The *green layer* of bark as shown in twigs or branches of *Forsythia*, cherry, alder, box elder, wahoo, or willow.

G. The white, fibrous inner layer, known as *hard bast*, of the bark of elm, leatherwood, or basswood.

27. Minute structure of the ordinary dicotyledonous stem. Cut thin cross sections of the stem of one of the perennial species of sunflower (*Helianthus*), or any large composite. Stain by immersing for a few seconds in a half-saturated aqueous solution of safranin, then wash, and examine in water, first with l.p. and then with m.p. The structural elements of the stem are considerably differentiated by the stain, the outer layers of the cortex appearing yellowish brown, the hard bast magenta, the wood fibers reddish magenta, and the pith salmon color.

References. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenck, Karsten, 1.

28. Minute structure of the climbing dicotyledonous stem.* *

A. Study, first with l.p. and then with m.p., thin cross sections of clematis stem[1] cut before the end of the first season's growth. Sketch the whole section without much detail, and then make a detailed drawing of a sector running from center to circumference and just wide enough to include one of the large bundles. In general label these drawings, as in Figs. 57 and 58 of the *Principles*. Note:

1. The general outline of the section.
2. The number and arrangement of the bundles. (How many kinds of bundles are there?)
3. The comparative areas occupied by the woody part of the bundle, and that which belongs to the bark.
4. The way in which the pith and the outer bark are connected (and the bundles separated) by the *medullary rays*.

B. Examine a longitudinal section of the same kind of stem to find out more accurately of what kinds of cells the pith, the bundles, and the outer bark are built. Which portion has cells that are nearly equal in shape, as seen in both sections?

REFERENCES. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenk, Karsten, 1.

29. Kinds of cells which compose stems.[2] Examine with m.p. these preparations (A–J below). Study very carefully each of the required sections, find in it the kind of cell referred to, and make a good drawing of a group of cells of each kind.

A. Very thin sections of the outside layers of the cortex of a potato, some cut tangential to the outer surface, other sections cut at right angles to it (*cork*).

B. Thin sections of the green layer of the bark of *Forsythia*, *Euonymus*, or box elder (*Negundo*) (*green cells of cortical parenchyma*).

C. Thin cross sections and lengthwise sections of the inner bark of linden twigs. Test with phloroglucin (*hard bast*).[3]

[1] *Clematis virginiana* is simpler in structure than some of the other woody species. *Aristolochia* or *Memispermum* sections will do very well. If unmounted sections are studied, stain with phloroglucin (Sec. 12, D). [2] See also Sec. 138, B.

[3] Both hard-bast fibers and wood fibers are known as *sclerenchyma*, but they differ somewhat in appearance and much in location.

D. Lengthwise sections of the stem of squash or cucumber plants (*sieve cells* or *soft bast*).

E. Thin cross sections of young twigs of pine or oak, collected and preserved in late summer (*cambium*).

F. Thin cross sections and lengthwise sections of apple, plum, maple, or box-elder wood. Test with phloroglucin (*wood fibers*).[1]

G. Thin lengthwise sections of any coniferous wood. Test with phloroglucin (*tracheids*).

H. Thin lengthwise sections of the stem of castor-oil plant (*Ricinus*) or of banana fruit stalks (*vessels*).

I. Thin lengthwise radial sections of sycamore, sassafras, or red-cedar wood (*wood parenchyma*).

J. Thin sections of pith of the stem of elder or sunflower (*pith cells*).

REFERENCES. Strasburger-Hillhouse, 6; Strasburger, Noll, Schenck, Karsten, 1.

30. Comparative structure of monocotyledonous and dicotyledonous bundles.* Examine with a power of about 150 diameters:

A. The cross section of a bundle of the corn stem stained with phloroglucin.

B. The cross section of a bundle of *Aristolochia* stem,[2] stained with phloroglucin.

Decide by referring to your drawings in Secs. 25, 28, which is the outer part of each bundle. Observe the number and position of the area made up of lignified fibers (stained by the phloroglucin), the cambium (in B), and the sieve tubes. These tubes are less easy to identify than most of the other elements of the bundles, but may be known by their location: in A, partly between but mostly outward (toward the rind) from the pair of large vessels; in B, just outside the cambium of the bundle. Note the general resemblance between the two kinds of bundles, with the presence of cambium in B as much the most important point of difference between them.

31. The dicotyledonous stem, thickened by secondary growth.

A. Cut off, as smoothly as possible, a small branch of hickory and one of white oak above and below each of the rings of scars already mentioned, and count the rings of wood above and below each ring of scars. How do the numbers correspond? What does this indicate?

[1] Both hard-bast fibers and wood fibers are known as *sclerenchyma*, but they differ somewhat in appearance and much in location.

[2] This section should be made from a young stem collected and preserved during the early part of the summer.

B. Count the rings of wood on the cut-off ends of large billets of some of the following woods: locust, chestnut, sycamore, oak, hickory. Do the successive rings of the same tree agree in thickness? Why or why not? Does the thickness of the rings appear uniform all the way round the stick of wood? If not, the reason in the case of an upright stem (trunk) is perhaps that there was a greater spread of leaves on the side where the rings are thickest (*Principles*, Fig. 76). Plant food, in the case of trees, is mainly produced in the leaves, and the course through the trunk of sugar or other food in solution is mainly straight down along the sieve tubes of the young wood. This would account for more rapid growth on the more leafy side. Sometimes the inequality may be because there was unequal pressure caused by bending before the wind. Do the rings of any one kind of tree agree in thickness with those of all the other kinds? What does this show?

C. In all the woods examined look for:

1. Contrasts in color between the heartwood and the sapwood.
2. The narrow lines running, in very young stems, pretty straight from pith to bark; in older wood extending only a little of the way from center to bark,—the *medullary rays*.
3. The wedge-shaped masses of wood between these.
4. The pores which are so grouped as to mark the divisions between successive rings. These pores indicate the cross sections of *vessels* or *ducts*. Note the distribution of the vessels in the rings to which they belong, and decide at what season of the year the largest ducts are mainly produced. Make a careful drawing of the end section of one billet of wood, natural size.

D. Cut off a grapevine several years old and notice the great size of the vessels.

E. Examine the smoothly planed surface of a billet of red oak that has been split through the middle of the tree, and note the large, shining plates formed by the medullary rays.

WORK OF THE STEM

EXPERIMENT XXII

Course of water in stems.* *

A. Cut some short branches from an apple tree or a cherry tree, and stand the lower end of each in eosin solution; try the same experiment with twigs of oak, ash, or other porous wood, and after some hours [1] examine with the lens and with the microscope, using l.p., successive cross sections of one or more twigs of each kind. Note exactly the portions through which the eosin has traveled. Pull off the leaves from one of the stems after standing in the eosin solution, and notice the spots on the leaf scar through which the eosin has traveled. These spots show the positions of the *leaf traces*, or fibro-vascular bundles, connecting the stem and the leaf.

B. Repeat with several potatoes cut crosswise through the middle.

C. Try also some monocotyledonous stems, such as those of the lily or asparagus.

D. For the sake of comparison between roots and stems treat any convenient root, such as a parsnip, in the same way.

Examine the longitudinal sections of some of the twigs, the potatoes, and the roots. In drawing conclusions about the channels through which the eosin has risen (those through which the newly absorbed soil water most readily travels), bear in mind the fact that a slow soakage of the eosin will take place in all directions, and therefore pay attention only to the strongly colored spots or lines.

What conclusions can be drawn from this experiment as to the course followed by the soil water?

REFERENCES. Detmer-Moor, 9; Ganong, 10; Strasburger, Noll, Schenck, Karsten, 1; Pfeffer-Ewart, 31, I.

[1] If the twigs are leafy and the room is warm, only from five to thirty minutes may be necessary. The experiment may be performed with a translucent-stemmed plant like *Impatiens Sultani*, and the course of the eosin watched. See Ganong, 10.

EXPERIMENT XXIII

What effect does loss of water have on the firmness of plant tissues? How long does it take for the water to be restored?

A. Allow a fuchsia or a hydrangea[1] which is growing in a flowerpot to wilt considerably for lack of water.

B. Then water it freely and record the time required for the leaves to begin to recover their natural position and the time to recover fully. The time needed for the leaves to begin to resume their ordinary position is that consumed in entering the roots (largely through the root hairs) and pushing upward through the stem until the water pressure in the leaves is restored to its normal amount. Filling the leaf cells fuller of water (increasing their turgor) has the same effect on their firmness that inflating a football or a bicycle tire does upon its firmness.

REFERENCE. Pfeffer 31, I.

32. Examination of twigs for starch. Cut thin cross sections of twigs of some common deciduous tree or shrub in its early winter condition, moisten with iodine solution, and examine for starch with a moderately high power of the microscope. Sketch the section with a pencil, coloring faintly the starchy portions with blue ink, used with a mapping pen, and describe exactly in what portions the starch is deposited.

33. A typical tuber: the potato. Sketch the general outline of a potato, showing the attachment to the stem from which it grew.[2]

A. Note the distribution of the "eyes." Are they opposite or alternate? Examine them closely with the magnifying glass and then with the lowest power of the microscope. What do they appear to be?

B. If the potato is a stem, it may branch; look over a lot of potatoes to try to find a branching specimen. If such a one is secured, sketch it.

[1] *Hydrangea Hortensia.*

[2] Examination of a lot of potatoes will usually discover specimens with an inch or more of attached stem.

C. Note the little scale overhanging the edge of the eye, and see if you can ascertain what this scale represents.

D. Cut the potato across, and notice the faint broken line which forms a sort of oval figure some distance inside the skin. Place the cut surface in eosin solution, allow the potato to stand so for many hours, and then examine, by slicing off pieces parallel to the cut surface, to see how far and into what portions the solution has penetrated. Refer to the notes on the study of the parsnip (Sec. 21), and see how far the behavior of the potato treated with eosin solution agrees with that of the parsnip so treated.

E. Cut a thin section at right angles to the skin, and examine with a high power. Moisten the section with iodine solution and examine again.

F. If possible, secure a potato which has been sprouting in a warm place for a month or more (the longer the better), and look near the origins of the sprouts for evidences of the loss of material from the tuber.

EXPERIMENT XXIV

Use of cork.* * Carefully weigh a potato; then pare another larger one, and cut portions from it until its weight is made approximately equal to that of the first one. Expose both freely to the air for some days and reweigh. What does the result show in regard to the use of the corky layer of the epidermis?

34. Structure of a bulb; the onion.

A. Examine the external appearance of the onion, and observe the thin membranaceous skin which covers it. This skin consists of the broad sheathing bases of the outer leaves which grew on the onion plant during the summer. Remove these and notice the thick scales (also formed from bases of leaves) which make up the substance of the bulb.

B. Make a transverse section of the onion at about the middle, and sketch the rings of which it is composed. Cut a thin

section from the interior of the bulb, examine with a moderate power of the microscope, and note the thin-walled cells of which it is composed.

C. Split another onion from top to bottom and try to find:

1. The broad flattened stem inside at the base.
2. The central bud.
3. The bulb scales.
4. In some onions (particularly in large, irregular ones) the bulblets, or side bulbs, arising in the axes of the scales near the base.

D. Test the cut surfaces for starch.

EXPERIMENT XXV

Testing for reserve sugar in an onion. Boil some slices of onion in a little water and filter the latter through a paper filter to remove bits of the bulb that may be left in it. Add a little Fehling's solution to the liquid thus obtained and heat to boiling. Result?[1] What is proved?

EXPERIMENT XXVI

Testing an onion for proteids. Heat a rather thick slice of onion in a porcelain evaporating dish with a little strong nitric acid until the latter just begins to boil.[2] Pour off the excess of acid, rinse the portion of onion for a moment with water, and add enough ammonia to cover it. Note any color changes. What is proved?

BUDS

35. Dissection of the horse-chestnut bud.[3] * * Examine one of the lateral buds on a twig in its winter or early spring condition.

A. Make a sketch of the external appearance of the bud as seen with a lens. How are the scales arranged? Notice the sticky coating upon them.

[1] The mixture usually blackens at length, probably owing to the presence of sulphur in the onion.

[2] Do not allow the acid to touch the hands or the clothing.

[3] Buds of buckeye, maple, or box elder will answer, but not as well. They may be forced to open early by placing twigs in water in a warm room for several weeks.

B. Remove the scales in pairs, arranging them thus:

How many pairs are found?

As the scales are removed observe whether the sticky coating is thicker on the outside or the inside of each scale, and whether it is equally abundant on all the successive pairs. What do you suppose to be the probable use of this coating? Note the delicate veining of some of the scales as seen through the magnifying glass. What does this mean? Inside the innermost pair are found two forked, woolly objects. What are these? Their shape could be more readily observed if the woolly coating were removed. Can you suggest a use for the woolly coating?

C. Examine a terminal bud in the same way in which you have just studied the lateral bud. It may contain parts not found in the other. What is the appearance of these parts? What do they represent? If there is any doubt about their nature, study them further on a horse-chestnut tree during and immediately after the process of leafing out in spring, or let the twigs remain in water for a few weeks until the buds open.

D. For comparison study at least one of the following kinds of buds in their winter or early spring condition: hickory, butternut, beech, ash, magnolia (or tulip tree), lilac, balm of Gilead, cottonwood, cultivated cherry.[1]

REFERENCE. Ganong, 7.

36. Study of a cabbage (a naked bud). Examine and sketch a rather small, firm cabbage, preferably a red one, which has been split lengthwise through the center, and note:

A. The short, thick, conical stem.

B. The crowded leaves which arise from the stem, the lower and outer ones largest and most mature, the upper and innermost ones the smallest of the series.

C. The axillary buds, found in the angles made by some leaves with the stem.

37. Study of vernation. Procure a considerable number of buds which are just about to burst, and others which have begun to open. Cut each across with a razor or very sharp scalpel; examine first with a magnifying glass, and then with the lowest power of the microscope. Make a careful sketch of one section. Pick to pieces other buds of the same kinds under the magnifying glass, and report upon the manner in which the leaves are packed away.

REFERENCE. Kerner-Oliver, 2.

38. The growing apex of the stem. The tip of the stem consists of tissue which is undergoing (or in resting buds is ready to undergo) rapid cell division, thus continuing the growth of the stem. The structure of this region in dicotyledons is difficult to make out and it is not recommended that beginners should undertake to study it.

Hippuris.[2] Choose a stem with a strong terminal bud, and trim away from near the tip all the larger leaves. Cut off about a third of an inch of the tip of the stem, hold it point downward between the thumb and forefinger, and try to get a smooth longitudinal section through the axis of the bud. If the latter is first split into halves and then successive sections are cut from each half, some one may be found to have passed exactly through

[1] If some of the buds are studied at home, pupils will have a better chance to examine at leisure the unfolding process.

[2] If *Hippuris* is not available, *Myriophyllum*, which grows readily in aquaria the year round, may be substituted.

the middle of the bud.[1] The cell contents may, in great part, be removed, and the sections rendered more transparent by treating them for some time with strong potash solution, which is then to be washed out, and the sections placed in concentrated acetic acid. They may be examined in acetic acid or in a solution of potassium acetate.

Examine the sections with a power of 200–300 diameters and make out the way in which the growing apex of the stem is capped by a series of layers of cells as follows:

A. On the outside a single layer, the *dermatogen*, from which the epidermis is developed.

B. Beneath this the *periblem*, four layers of cells from which the bark, or *cortex*, is formed.

C. Within the layers of the periblem, the *plerome*, out of which the axial fibro-vascular bundle of the stem is for the most part formed.

Make a drawing to show the relations of all the parts above described, and (lower down on the stem) the origins of the leaves.

LEAVES

39. The elm leaf.[2] * *

A. Sketch the leafy twig of elm that is supplied to you. Report on the following points:

1. How many rows of leaves?
2. How much overlapping of leaves when the twig is held with the upper sides of the leaves toward you? What would be the advantages or disadvantages of much overlapping? Are the spaces between the edges of the leaves large or small compared with the leaves themselves?

B. Pull off a single leaf and make a sketch of its under surface, about natural size. Label the broad part the *blade*, the stalk by which it is attached to the twig *leafstalk* or *petiole*, the appendages at its base *stipules*. Study the outline of the leaf and answer these questions (see *Principles*, Appendix):

1. What is the shape of the leaf as a whole?

[1] Unless the students have had considerable practice in making sections it will be better to purchase slides of microtome sections of some growing point.

[2] If this subject is taken up during the winter, it will be necessary to use geranium or other leaves from the florists for the study of leaf anatomy, and potted geraniums, begonias, lilies, etc., for leaf arrangement.

2. Is the leaf *bilaterally symmetrical;* i.e. is there a middle line running through it lengthwise, along which it could be so folded that the two sides would nearly coincide?
3. Is the leaf *dorsiventral;* i.e., has it distinct upper and under surfaces?
4. Notice that the leaf is traversed lengthwise by a strong *midrib* and that many so-called *veins* run from this to the margin. Are these veins parallel? Hold the leaf up towards the light and see how the main veins are connected by smaller *veinlets.* Examine with your glass the leaf as held to the light, and make a careful sketch of portions of one or two veins and the intersecting veinlets. How is the course of the veins shown on the upper surface of the leaf?
5. Examine both surfaces of the leaf with the glass and look for hairs distributed on the surfaces. Describe the manner in which the hairs are arranged.

40. The maple leaf.

A. Sketch the leafy twig.

1. How are the leaves arranged?
2. How are the petioles distorted from their natural positions to bring the proper surface of the leaf upward toward the light?
3. Do the edges of these leaves show larger spaces between them than the elm leaves did; i.e. would a spray of maple intercept the sunlight more or less perfectly than a spray of elm? Pull off a single leaf and sketch its lower surface, about natural size.
4. Of the two main parts (blade and petiole), which is more developed in the maple than in the elm leaf?

B. Describe:

1. The shape of the maple leaf as a whole. To settle this, place the leaf on paper, mark the positions of the extreme points, and connect these by a smooth line.

2. Its outline as to main divisions. Of what kind and how many?
3. The detailed outline of the margin.

Compare the mode of veining, or *venation*, of the elm and the maple leaf by making a diagram of each (see *Principles*, Chapter X).

The leaves of elm and of maple agree in being *netted veined*, i.e. in having veinlets that join each other at many angles, so as to form a sort of delicate lace work.

Such a leaf as that of the elm is said to be feather veined, or *pinnately veined*. The maple leaf, or any leaf with closely similar venation, is said to be *palmately veined*. Describe the difference between the two plans of venation.

LEAF ARRANGEMENT FOR EXPOSURE TO LIGHT AND AIR; HELIOTROPIC MOVEMENTS OF LEAVES AND SHOOTS

EXPERIMENT XXVII

Is the nocturnal position due to removal of the light stimulus or to other causes? Remove a pot containing an oxalis or a clover plant from a sunny window to a dark closet, at about the same temperature, and note at intervals of five minutes the condition of its leaves for half an hour or more.

References. Darwin and Acton, 11; Pfeffer-Ewart, 31, III; Detmer-Moor, 9.

EXPERIMENT XXVIII

Determination of the values of illumination to produce various leaf positions.[1] Select a few common bean plants (*Phaseolus*) growing vigorously in a sunny place, or a locust tree (*Robinia*) at the time in the spring when its leaves have just reached their full size.

A. Note and sketch the positions of the leaves as follows:

1. After dusk.
2. In cloudy daylight or near dusk.
3. In intense sunlight, near noon.

[1] This is preferably an out-of-door study.

B. Determine the relative proportion of the maximum illumination of sunlight needed to bring about positions 1, 2, and 3. The light measurements are to be made by means of ordinary photographic printing paper ("solio" paper answers well) as follows: cut the paper in a very dark room into pieces about an inch square and at once put them into small pasteboard or tin boxes and shut them away in a close drawer or a windowless closet. *All the paper in each box must be cut from the same sheet of sensitive paper.* One square of paper may be marked with a violet aniline pencil and then exposed, at about noon, to the rays out of doors, so that they will strike it vertically. Note exactly with a watch in how many seconds the pencil mark nearly disappears. The paper should then be at once shut up in the box from which it was taken. This darkened square of paper may now be used as a standard. If it darkened in 30 seconds, and another square used to measure illumination (1) darkened to the same tint in 2400 seconds, then illumination (1) was $\frac{1}{80}$ full sunlight, or 1.25 per cent.

RECORD

Highest illumination for position 1 .....
Average illumination for position 2 .....
Least illumination for position 3 .....

What is the apparent object of these movements? What other plants have as many positions as the bean and the locust?

Reference, Pfeffer-Ewart, 31, III.

EXPERIMENT XXIX

Can growing leaves adapt their positions to new light relations? * * Select a young, leafy, vertical branch of maple growing out of doors, or a vigorous young sunflower (*Helianthus*) plant growing out of doors or under nearly vertical light.[1] Bend the shoot into a horizontal position and note whether the leaves adapt themselves to their new relations. If there is any adaptation, describe exactly the leaf movements by which it is brought about.

Reference. Pfeffer-Ewart, 31, III.

[1] Less satisfactory studies can be made of geraniums, begonias, or other plants grown in the window and turned at intervals of several weeks.

EXPERIMENT XXX

How do young shoots of English ivy bend with reference to light? Place a thrifty potted plant of English ivy before a small window, e.g. an ordinary cellar window, or in a large covered box, painted dull black within and open only on the side toward a south window. After some days note the position of the tips of the shoots. Explain the use to the plant of their movements.

REFERENCE. Detmer-Moor, 9.

41. Sun leaves and shade leaves.[1] Select for study some species of shrub or tree which furnishes a dense shade. Deciduous species will answer, but broad-leafed evergreens, like hollies, some rhododendrons, or live oaks are still better. Why? Gather some of the outer leaves and some of the innermost ones *from the same tree.* Measure the per cent of total illumination received by the innermost leaves, as described in Exp. XXVIII. Make a detailed comparison of the two kinds of leaves (those grown in sun and in shade) as follows:

A. Comparison of average areas (see Exp. XXXVIII).

B. Comparison of hairiness or scaliness of the under surfaces.

C. Comparison of thickness of leaves. Use a power of 25–50 diameters and a micrometer eyepiece, if one is available.

D. Comparison of details of structure of cross sections (see Secs. 42, 43). Explain as fully as possible all the differences noted in comparisons A–D above.

REFERENCE. Clements, 59.

MINUTE STRUCTURE OF LEAVES; FUNCTIONS OF LEAVES

42. Minute structure of lily leaf.[2] * *

A. The student should first examine with m.p. a cross section of the leaf. This will show:

1. The upper epidermis of the leaf, a thin, nearly transparent membrane.
2. The intermediate tissues.
3. The lower epidermis.

[1] A simpler study may be made by comparing the illuminations and structures of characteristic sun plants and shade plants, for instance *Portulaca*, *Sedum*, etc., with *Arisæma*, *Aralia*, *Clintonia*, *Trillium*, etc., each grown in its natural habitat.

[2] Any kind of lily will answer. Other leaves are equally good but many of them are not obtainable at all seasons. Some excellent kinds are *Fuchsia*, English

In order to ascertain the relations of the parts, and to get their names, consult *Principles*, Fig. 112. Your section is by no means exactly like the figure; sketch it. Label properly all the parts shown in your sketch.

Are any differences noticeable between the upper and the lower epidermis? Between the layers of cells immediately adjacent to each? Test some sections with phloroglucin (Sec. 12, D).

B. Examine with a power of 200 or more diameters the outer surface of a piece of epidermis from the lower side of the leaf.[1] Sketch carefully, comparing your sketch with *Principles*, Fig. 113, and labeling it to agree with that figure.

C. Examine another piece from the upper surface; sketch it. How does the number of stomata in the two cases compare?

REFERENCE. Strasburger-Hillhouse, 6.

43. Study of the leaf of "rubber plant" (*Ficus elastica*).* *

A. Make preparations of the leaf of the so-called rubber plant as already described for the lily leaf. Study and sketch them and then compare the two types of leaf:

1. As regards thickness of epidermis.
2. As regards number of layers of cells in the epidermis.
3. As regards development of the palisade layers.
4. As regards amount of fibro-vascular material (veins).
5. As regards freedom of exposure of the stomata openings to the air.

ivy (*Hedera*), willow, maple, poplar (any species, as cottonwood, aspen, etc.), the thicker-leaved species of aster, apple, pear, plum, quince, beet. Thin sections may be cut free-hand, especially if the leaf is doubled together several times or held between two bits of elder pith. If only a part of the section is very thin, it will answer almost as well as if it were equally thin throughout. The sections may be made much more transparent if they are soaked in potash solution until most of the green color disappears, and then treated with acetic acid. Both these sections and those in their natural condition should be examined. Some sections in their natural condition should be treated with phloroglucin.

[1] The epidermis may be started with a sharp scalpel and then peeled off with small forceps and mounted in water for microscopical examination. The epidermis of *Ficus* leaf (Sec. 43) will need to be pared off with a very sharp razor held parallel to the leaf surface. The stomata may be counted by use of an eyepiece micrometer ruled in squares. Find how many divisions of the stage micrometer equal one side of this square; then substitute a bit of epidermis for the stage micrometer, and count the number of stomata in an eyepiece square. Calculate the number of stomata in a leaf of the kind examined; also, if possible, the number for the entire plant.

B. Let an entire leaf of each kind remain for some hours in a warm, sunny place and notice the comparative amount of wilting in both cases. Explain.

REFERENCES. Kerner-Oliver, 2; Haberlandt, 33; Schimper-Fisher, 56; Warming-Graebner, 57.

EXPERIMENT XXXI

Oxygen making in sunlight.** Place a green aquatic plant in a glass jar full of water, at about 70° F. (21° C.), in front of a sunny window.[1] Note the formation of oxygen bubbles looking silvery by reflected light.[2] Remove to a dark closet and after fifteen minutes examine by lamplight, to see whether the rise of bubbles still continues.

This gas may be shown to be oxygen by collecting some of it in a small inverted test tube filled with water and thrusting into it the glowing coal of a match just blown out. It is not, however, always very easy to do this satisfactorily.

Repeat the experiment, using water which has been well boiled and then quickly cooled in a tightly covered vessel. Boiling removes all the dissolved gases from water (including much carbon dioxide), and they are not redissolved in any considerable quantity for many hours.

REFERENCES. Detmer-Moor, 9; Darwin and Acton, 11.

EXPERIMENT XXXII

Occurrence of starch in nasturtium leaves.** Toward the close of a very sunny day collect some bean leaves or leaves of nasturtium (*Tropæolum*). Boil these in water for a few minutes, to kill the protoplasmic contents of the cells and to soften and swell the starch grains. Soak the leaves, after boiling, in strong, hot alcohol for half an hour, to dissolve out the chlorophyll, which

[1] *Elodea*, *Myriophyllum*, *Chrysosplenium*, *Potamogeton*, any of the green aquatic flowering plants, the aquatic moss, *Fontinalis*, or even the common pond scum, *Spirogyra*, will do for this experiment.

[2] Some of the earlier bubbles may contain a good deal of air which was dissolved in the water and set free as it grows warm, but the later bubbles will be fairly pure oxygen.

might obscure the starch test. Heat the alcohol in a water bath away from any flame. Place the leaves for ten or fifteen minutes in a solution of iodine, rinse off with water, put in a white plate or saucer, and note what portions of the leaf, if any, show the presence of starch.

REFERENCES. Detmer-Moor, 9; Ganong, 10; Darwin and Acton, 11; Pfeffer-Ewart, 31, I.

EXPERIMENT XXXIII

Consumption of starch in nasturtium (Tropæolum) leaves.* * Select some healthy leaves of *Tropæolum* on a plant growing vigorously indoors, or, still better, in the open air. Shut off the sunlight from parts of the selected leaves (which are to be left on the plant and as little injured as possible) by pinning circular disks of cork loosely on opposite sides of the leaf, as shown in Fig. 3. On the afternoon of the next day remove from the plant these leaves and (for control purposes) some others to which no cork disks were attached. Treat all as described in the preceding experiment, taking especial pains to get rid of the chlorophyll by changing the alcohol as many times as may be necessary. What does this experiment show in regard to the consumption of starch in the leaf? What has caused its disappearance?[1]

FIG. 3. Leaf of *Tropæolum* partly covered with disks of cork and exposed to sunlight

It may be fairly taken for granted that if the leaf contained any starch when the corks were pinned onto it, all parts of it were somewhat equally full of starch. If the experiment results in showing absence of starch in the part deprived of light by the cork, it may be thought that starch manufacture was stopped

[1] Or put a plant with starch in the leaves in a moist chamber without light for a day or two, with cut-off leaves beside it. Then test the attached leaves and the cut-off ones for starch. Explain results.

in that portion partly because of lack of light, and partly because the supply of air (and therefore of carbon dioxide) was very scanty under the cork. The truth of the supposition that lack of carbon dioxide was responsible for the failure to make starch may be tested by boring a large hole with a cork borer through each cork before fastening it in place on the leaf, and cementing over the hole a thin cover glass. Then some of the parts of the leaf covered by the cork are lighted while others are not, and if the lighted parts show starch, it was lack of light only that prevented its formation in the shaded parts.

REFERENCES. (See Experiment XXXII.)

EXPERIMENT XXXIV

Can starch making go on when the stomata are shut off from all air supply? Select a thrifty potted plant of some species which has thin leaves, with stomata only on the under surface (e.g. primrose, begonia). Put the plant in an absolutely dark place for twenty-four hours, and then coat half of the under surface of one or more leaves with vaseline and expose the plant for a day to bright sunlight. Wipe off most of the vaseline with cotton wool and remove the rest by washing, with a swab or soft brush, in several successive quantities of benzine.[1] Then boil, treat with alcohol, and test for starch as directed in Exp. XXXII. Explain the result.

REFERENCE. Ganong, 10.

EXPERIMENT XXXV

Can squash seedlings make chlorophyll in the dark? * * Plant some squash seeds in sawdust or sand, and keep part of them in a good light, while others are kept in total darkness at about the same temperature. When the plumules of those in the light are developed into half-grown leaves, sketch both lots of seedlings

[1] Do not attempt this in the same room with a flame, or a lighted lamp or gas jet.

and describe the main differences in color, height, and thickness of hypocotyl, and in the development of the cotyledons of those grown in darkness and in the light.

What is the conclusion in regard to power to make chlorophyll?

Leave both lots in sunlight for a day and test some cotyledons of each set for starch. Leave both sets in sunlight for several days more and note any changes in the appearance of those which were started in darkness. Test the latter again for starch. Conclusions?

REFERENCE. Pfeffer-Ewart, 31, II.

EXPERIMENT XXXVI

Do leaves give off water? If so, from which surface is it given off more abundantly? * * Fasten two small watch glasses, one on each side of a leaf of a plant growing vigorously in a pot or out of doors.

FIG. 4. Watch glasses fastened on a leaf of Chinese primrose

Hydrangea,[1] primrose, or cineraria[2] are good plants for the purpose, although many others will answer. The watch glasses may be held in place by a spring clip, as shown in Fig. 4. Seal the margin of each glass all the way around by means of vaseline or barely melted grafting wax. Leave the plant for half an hour or more in a sunny place, and then look for drops of water inside

[1] *H. Hortensia.* [2] *Senecio cruentus.*

of each watch glass. If none are visible, carefully cut off the leaf and place it for a few minutes in a box with a piece of ice or put it out of doors in a cold place. Report the results. Examine the upper and lower epidermis with the microscope and explain the results noted.

REFERENCE. Osterhout, 13.

EXPERIMENT XXXVII

Through which side of a leaf of Ficus elastica does transpiration occur? The student may already have found (Sec. 43) that there are no stomata on the upper surface of the *Ficus* leaf which he studied.[1] That fact makes this leaf an excellent one for the study of the relation of stomata to transpiration.

Take two large, sound *Ficus* leaves, cut off pretty close to the stem of the plant. Slip over the cut end of the petiole of each leaf a piece of small rubber tubing, wire this on, leaving about half of it free, and then double the free end over and wire tightly, so as to make the covering moisture proof. Warm some vaseline or grafting wax until it is almost liquid, and spread a thin layer of it smoothly over the upper surface of one leaf and the lower surface of the other. Hang both up in a sunny place in the laboratory and watch them for a month or more.

What difference in the appearance of the two leaves becomes evident? What does the experiment prove?

REFERENCE. Darwin and Acton, 11.

EXPERIMENT XXXVIII

Amount of water lost by transpiration. * * Procure a thrifty hydrangea[2] and a small plant of *Ficus elastica*,[3] each growing in a small flowerpot, and with the number of square inches of leaf surface in the two plants not too widely different. Calculate the area of the leaf surface for each plant by dividing the surface of a piece of tracing cloth into a series of squares one half inch on a side, holding an average leaf of each plant against this and counting the number of squares and parts of squares covered by the leaf. This area, multiplied by the number of leaves for each plant, will give approximately the total evaporating surface for each.[4]

[1] This is also true of many other leaves, as those of the oleander, the lilac, and most begonias, and any of them may be used for the experiment.

[2] The common species of the greenhouse, *Hydrangea Hortensia*.

[3] Commonly known as India-rubber plant.

[4] The quickest and most accurate method of procedure is to defer calculating the leaf area until the conclusion of the experiment, and then to cut off all the

Transfer each plant to a glass battery jar of suitable size. Cover the jar with a piece of thin sheet lead, slit to admit the stem of the plant, invert the jar, and seal the lead to the glass with a hot mixture of beeswax and rosin. Seal up the slit and the opening about the stem with grafting wax.[1] A thistle tube, such as is used by chemists, is also to be inserted, as shown in Fig. 5. The mouth of this may be kept corked when the tube is not in use for watering.

FIG. 5. A hydrangea potted in a battery jar for Exp. XXXVIII

Water each plant moderately and weigh the plants separately on a balance that is sensitive to one-fifth gram. Record the weights, allow the plants to stand in a sunny, warm room for twenty-four hours, and reweigh.

Add to each plant just the amount of water which is lost,[2] and continue the experiment in the same manner for several days, so as to ascertain, if possible, the effect upon transpiration of varying amounts of water in the atmosphere.

Calculate the average loss per 100 square inches of leaf surface for each plant throughout the whole course of the experiment. Divide the greater loss by the lesser to find the ratio. Find the ratio of each plant's greatest loss per day to its least loss per day, and by comparing these ratios decide which transpires more regularly.

Try the effect of supplying very little water to each, so that the hydrangea will begin to droop, and see whether this changes the relative amount of transpiration for the two plants. Vary the conditions of the experiment for a day or two as regards temperature, and again for a day or two as regards light, and note the effect upon the amount of transpiration.

The structure of the *Ficus* (India-rubber plant) leaf has already been studied. That of the hydrangea is looser in texture and more like the leaf of the lily.

leaves, make blue prints of them, cut these out, and weigh them. The total area may easily be calculated by comparison of the weight obtained with that of a known area of the paper used.

[1] It will be much more convenient to tie the hydrangea, if one has been chosen that has but a single main stem. Instead of the hydrangea the common cineraria, *Senecio cruentus*, or a small sunflower plant does very well.

[2] The addition of known amounts of water may be made most conveniently by measuring in a cylindrical graduate.

What light does the structure throw on the results of the preceding experiment?

REFERENCES. Detmer-Moor, 9; Ganong, 10.

EXPERIMENT XXXIX

Passage of water from stem to leaf. Place a freshly cut leafy shoot of some plant with large, thin leaves, such as *Hydrangea Hortensia*, in eosin solution for a few minutes. As soon as the leaves show a decided reddening pull some of them off and sketch the red stains on the scars thus made. What does this show?

EXPERIMENT XL

Rise of water in leaves.* * Put the freshly cut ends of the petioles of several thin leaves of different kinds into small glasses, each containing eosin solution to the depth of one quarter inch or more. Allow them to stand for half an hour, and examine them by holding up to the light and looking through them to see into what parts the eosin solution has risen. Allow some of the leaves to remain as much as twelve hours, and examine them again. The red-stained portions of the leaf mark the lines along which, under natural conditions, water rises into it. Cut across (near the petiole or midrib ends) all the principal veins of some kind of large, thin leaf. Then cut off the petiole and at once stand the cut end, to which the blade is attached, in eosin solution. Repeat with another leaf and stand in water. What do the results teach?

EXPERIMENT XLI

Does the leaf vary in its starch contents at different seasons? Collect in early summer, at the close of a sunny day, some leaves of different kinds of trees and shrubs and preserve them in alcohol. Collect other leaves of the species as they are beginning to drop from the trees in autumn and preserve them in the same way. Test some of each lot for starch, as described in Exp. XXXII.

What does the result indicate?

THE FLOWER OF THE HIGHER SEED PLANTS

44. The flower of the Trillium.[1] * * *

A. Cut off the flower stalk rather close to the flower; stand the latter, face down, on the table, and draw the parts then shown. Label the green leaf-like parts *sepals*, and the white parts, which alternate with these, *petals*.

B. Turn the flower face up and make another sketch, labeling the parts as before, together with the enlarged yellow extremities, or *anthers*, of the stalked organs called *stamens*.

C. Note and describe the way in which the petals alternate with the sepals. Observe the arrangement of the edges of the petals toward the base, — how many with both edges outside the others, how many with both edges inside, how many with one edge in and one out.

Note the veining of both sepals and petals, observing in which set they are more distinct.[2]

D. Pull off a sepal and make a sketch of it, natural size; then remove a petal, flatten it out, and sketch it, natural size.

E. Observe that the flower stalk is enlarged slightly at the upper end into a rounded portion, the *receptacle*, on which all the parts of the flower rest.

F. Note how the six stamens arise from the receptacle, and their relations to the origins of the petals. Remove the remaining petals (cutting them off near the bottom with a

[1] Only one flower need be studied to give an idea of the floral organs ordinarily found. More advanced studies are suggested at the end of Part III.

If none of the three flowers here described can be had, the instructor can readily frame a set of directions for the examination of some other form. Among the simplest types which can readily be grown in the greenhouse for class study are *Sedum acre* and *Crassula quadrifida*. *Matthiola* is not quite so simple, but the single-flowered varieties answer very well. *Scilla sibirica* is often available. Another convenient greenhouse flower is the Roman hyacinth.

[2] In flowers with delicate white petals the distribution of the fibro-vascular bundles can usually be readily shown by standing the freshly cut end of the flower stalk in eosin for a short time, until colored veins begin to appear in the petals. The experiment succeeds readily with apple, cherry, or plum blossoms; with white gillyflower the coloration is very prompt. Lily of the valley is perhaps as interesting a flower as any on which to try the experiment, since the well-defined stained stripes are separated by portions quite free from stain, and the pistils are also colored.

knife), and sketch the stamens, together with the other structure, the *pistil*, which stands in the center.

Cut off one stamen, and sketch it as seen through the lens. Notice that it consists of a greenish stalk, the *filament*, and a broader portion, the *anther*. The latter is easily seen to contain a prolongation of the green filament, nearly surrounded by a yellow substance. In the bud it will be found that the anther consists of four long pouches, or *pollen chambers*, which are attached by their whole length to the filament. When the flower is fairly open the pollen chambers of each pair have already split down their margins, thus appearing as one on each side, and are discharging a yellow, somewhat sticky powder, the *pollen*.

Examine one of the anthers with a lens and sketch it. Cut thin cross sections of an immature anther and draw under l.p., showing the pollen chambers.

G. Cut away all the stamens and sketch the pistil. It consists of a stout lower portion, the *ovule case*, or *ovary*, which is six-ridged or angled, and which bears at its summit three slender *stigmas*.

In another flower, which has begun to wither (and in which the ovary is larger than in a newly opened flower), cut the ovary across about the middle, and with the lens determine the number of chambers, or *locules*, which it contains. Examine the cross section with the lens; sketch it, and note particularly the appearance and mode of attachment of the undeveloped seeds, or *ovules*, with which it is filled. Make a vertical section of another rather mature ovary, and examine this in the same way.

H. Using a fresh flower, construct a diagram to show the relation of the parts on an imaginary cross section.[1] Construct a diagram of a longitudinal section of the flower, showing the contents of the ovary.

[1] It is important to notice that such a diagram is not a picture of the section actually produced by cutting through the flower crosswise at any one level, but that it is rather a *projection* of the sections through the most typical part of each of the floral organs (see *Principles*, Fig. 138).

Make a tabular list of the parts of the flower, beginning with the sepals, giving the order of parts and the number in each set.

45. The flower of the tulip.[1]

A. Make a sketch of a side view of the well-opened flower as it appears when standing in sunlight. Observe that there is a set of outer flower leaves and a set of inner ones.[2] Label the outer set *sepals* and the inner set *petals*. In most flowers the parts of the outer set are greenish, and those of the inner set of some other color. It is often convenient to use the name *perianth* (meaning around the flower) for the two sets taken together. Note the white waxy bloom on the exterior surface of the outer segments of the perianth. What is the use of this? Observe the manner in which the inner segments of the perianth arise from the top of the flower stalk and their relation to the points of attachment of the outer segments. In a flower not too widely opened note the relative position of the inner segments of the perianth, how many wholly outside the other two, how many wholly inside, how many with one edge in and one edge out.

B. Remove one of the sepals by cutting it off close to its attachment to the peduncle, and examine the veining by holding it up in a strong light and looking through it. Make a sketch to show the general outline and the shape of the tip.

C. Examine a petal in the same way, and sketch it.

D. Cut off the remaining portions of the perianth, leaving about a quarter of an inch at the base of each segment. Sketch the upright, triangular, pillar-like structure in the center,—label it *pistil;* sketch the organs which spring from around its base, and label these *stamens.*

Note the fact that each stamen arises from a point just above and within the base of a segment of the perianth. Each stamen consists of a somewhat conical or awl-shaped portion below, the *filament*, surmounted by an ovate-linear portion, the *anther.*

E. Sketch one of the stamens about twice natural size and label it × 2. Is the attachment of the anther to the filament such as to admit of any nodding or twisting movement of the former? In a young flower note the tubular pouches, or pollen chambers, of which the anther is composed, and the slits by which these open. Observe the dark-colored *pollen* which escapes from the anther cells and adheres to paper or to the fingers. Examine a newly opened anther with the lens and sketch it. Cut thin cross sections of an unopened anther and examine with l.p. Note that there are four pollen chambers, two on each side.

[1] *Tulipa gesneriana.* [2] Best seen in a flower which is just opening.

F. Cut away all the stamens and note the two portions of the pistil,— the *ovule case*, or *ovary*, below, and above three roughened, scroll-like lobes of the *stigma*. Make a sketch of these parts about twice natural size, and label them × 2. Touch a small camel's-hair brush to one of the anthers and then transfer the pollen thus removed to the stigma. This operation is merely an imitation of the work done by insects which visit the flowers out of doors. Does the pollen cling readily to the rough stigmatic surface? Examine this adhering pollen under l.p. and sketch a few grains of it, together with the bit of the stigma to which it clings. Make a cross section of the ovary about midway of its length, and sketch the section as seen through the lens. Label the three chambers shown *locules*, and the white, egg-shaped objects within *ovules*.[1]

Make a longitudinal section of another ovary, taking pains to secure a good view of the ovules, and sketch as seen through the lens.

G. Making use of the information already gained and the cross section of the ovary as sketched, construct a diagram of a cross section of the entire flower, showing the contents of the ovary.

H. Split a flower lengthwise and construct a longitudinal section of the entire flower.

46. The flower of the buttercup.* *

A. Sketch the mature flower as seen in a side view, looking a little down into it. Label the pale greenish-yellow, hairy outermost parts, *sepals;* the larger, bright yellow parts above and within these, *petals;* and the yellow-knobbed organs which occupy a good deal of the interior of the flower, *stamens.*

B. Note the difference in the position of the sepals of a newly opened flower and that of the sepals of a flower which has opened as widely as possible. Note the way in which the petals are arranged in relation to the sepals. In an opening flower observe the arrangement of the edges of the petals,— how many entirely outside the others, how many entirely inside, how many with one edge in and the other out.

C. Cut off a sepal and a petal, each close to its attachment to the flower; place both, face down, on a sheet of paper, and

[1] The section will be more satisfactory if made from an older flower, grown out of doors, from which the perianth has fallen. In this case label the contained objects *developing seeds.*

sketch about twice the natural size and label it × 2. Describe the difference in appearance between the outer and the inner surface of the sepal and of the petal. Note the little scale at the base of the petal, inside. Lift up the free edge of this scale with the point of a needle and look for *nectar*.

D. Strip off all the parts from a flower which has lost its petals, until nothing is left but a slender, conical object a little more than an eighth of an inch in length. This is the *receptacle* or summit of the flower stalk.

E. In a fully opened flower note the numerous yellow-tipped *stamens*, each consisting of a short stalk, the *filament*, and an enlarged yellow knob at the end, the *anther*. Note the division of the anther into two portions, which appear from the outside as parallel ridges, but which are really closed cavities full of pollen.

F. Observe in the interior of the flower the somewhat globular mass (in a young flower almost covered by the stamens). This is a group of *pistils*. Study one of these groups in a flower from which the stamens have mostly fallen off, and make an enlarged sketch of the head of pistils. Remove some of the pistils from a mature head, and sketch a single one as seen with the magnifying glass. Label the little knob or beak at the upper end of the pistil *stigma*, and the main body of the pistil the *ovary*. Make a section of one of the pistils, parallel to the flattened surfaces, and note the partially matured seed within.

POLLINATION AND FERTILIZATION

EXPERIMENT XLII

Production of pollen tubes.* * Make a hanging-drop culture (Sec. 204), or place a few drops of suitably diluted sirup of cane sugar (Sec. 170), with some fresh pollen, in a concave cell ground in a microscope slide, and cover with a thin glass circle. Place the slide under a bell glass, with a wet cloth or sponge, to prevent

evaporation of the water, and set aside in a warm place, or merely put some pollen in sirup in a watch crystal under the bell glass. Examine from time to time to note the appearance of the pollen tubes. Try several kinds of pollen if possible, using solutions of various strengths. The following kinds of pollen form tubes readily in sirups of the strengths indicated :

Tulip	1 to 3 per cent
Narcissus	3 to 5 per cent
Cytisus canariensis (called Genista by florists)	15 per cent
Chinese primrose	10 per cent
Sweet pea	10 to 15 per cent
Tropæolum	15 per cent[1]

REFERENCE. Strasburger-Hillhouse, 6

THE FRUIT[2]

47. A capsule (legume), the bean pod.[3] * *

A. Lay the pod flat on the table and make a sketch of it, about natural size. Label *stigma*, *style*, *ovary*, *calyx*, *flower stalk*.

B. Make a longitudinal section of the pod, at right angles to the plane in which it lay as first sketched, and note the partially developed seeds, the cavities in which they lie, and the solid portion of the pod between each bean and the next. Split another pod, so as to leave all the beans lying undisturbed on one half of it, and sketch that half, showing the beans lying in their natural position and the *funiculus*, or stalk, by which each is attached to the *placenta*.

C. Make a cross section of another pod through one of the beans, sketch the section, and label the placenta. Break off sections of the pod and determine, by observing where the

[1] The sweet-pea pollen and that of *Tropæolum* are easier to manage than any other kinds of which the authors have personal knowledge. If a concave slide is not available, the cover glass may be propped up on bits of the thinnest broken cover glasses. From presence of air or for some other reason, the formation of pollen tubes often proceeds most rapidly just inside the margin of the cover glass.

[2] If time is not available for all of these studies, two or three types will suffice.

[3] Material in preservative fluid such as formalin will answer, or fresh string beans or shell beans may be used.

most stringy portions are found, where the fibro-vascular bundles are most numerous.

D. Examine some ripe pods of the preceding year,[1] and notice where the *dehiscence*, or splitting open of the pods, occurs, whether down the placental edge, *ventral suture*, the other edge, *dorsal suture*, or both.

48. A schizocarp, the fruit of caraway.[2] Examine a complete fruit, "caraway seed" (magnified). If it has not been roughly handled, it should show the remains of the stigmas, surmounting the two halves, *mericarps*, of the fruit. The mericarps are borne on a forked stalk, from which they remain suspended until blown away by the wind or otherwise detached. Make a cross section of one mericarp (if dry, after soaking it for a minute or two in hot water). Draw it magnified and label the pericarp, with its oil tubes and the seed within. The tubes contain the volatile oil which gives the fruit its characteristic smell and flavor.

This fruit has no very effective means for securing dispersal. Compare it in this respect with the fruits (commonly called seeds) of parsnip and of carrot.

49. An akene, the fruit of dock.

A. Hold in the forceps a ripe fruit of any of the common kinds of dock, and examine with the lens. Note the three dry, veiny, membranaceous sepals by which the fruit is inclosed. On the outside of one or more of the sepals is found a tubercle, or thickened appendage, which looks like a little seed or grain. Cut off the tubercles from several of the fruits; put these, with some uninjured ones, to float in a pan of water, and watch their behavior for several hours. What is apparently the use of the tubercle?

Of what use are the sepals after drying up? Why do the fruits cling to the plant long after ripening?

B. Carefully remove the sepals and examine the fruit within them. What is its color, size, and shape? Note the three tufted stigmas attached by slender threads to the apex of the fruit. What does their tufted shape indicate?

What evidence is there that this seed-like fruit is not really a seed?

[1] Preserved dry for the purpose.
[2] "Caraway seeds" can be bought from the druggists.

C. Make a cross section of a fruit and notice whether the wall of the ovary can be seen distinct from the seed coats. Compare the dock fruit in this respect with the fruit of the buttercup shown in *Principles*, Fig. 161. Such a fruit as either of these is called an *akene*.

50. A nut, the acorn.

A. Sketch the entire acorn, side view, with the base inclosed in its involucre, the "acorn cup." Note the remains of the stigma at the top of the acorn.

B. Cut a cross section of the acorn about midway of its length. Note the hard *pericarp* and the seed, with thick cotyledons.

C. Make a drawing of a lengthwise section of the seed cut at right angles to the surfaces where the cotyledons join. Look for the plumule and the hypocotyl. Note and describe the testa. Test the cotyledons for starch and for oil. Note the taste of the seeds. How are they disseminated?

If possible, compare the acorn with such other nuts as the chestnut and the hazelnut.

51. A berry, the tomato.

A. Study the external form of the tomato, and note the persistent calyx and peduncle.

B. Cut a cross section at about the middle of the tomato. Note the thickness of the epidermis (peel off a strip) and of the wall of the ovary. Note the number, size, form, and contents of the cells of the ovary. Observe the thickness and texture of the partitions between the cells. Sketch. What changes in the fruit of the pepper (*Principles*, Fig. 166) would make it resemble a tomato? Note the attachments of the seeds to the placentas, and the gelatinous, slippery coating of each seed.

The tomato is a typical berry, but its structure presents fewer points of interest than are found in some other fruits of the same general character, so the student will do well to spend a little more time on the examination of such fruits as the orange or the lemon.

52. A leathery-skinned berry, or hesperidium, the lemon. ** Procure a large lemon which is not withered; if possible, one which still shows the remains of the calyx at the base of the fruit.

A. Note the color, general shape, surface, remains of the calyx, knob at portion formerly occupied by the stigma. Sketch the fruit about natural size.

B. Examine the pitted surface of the rind with the lens, and sketch it.

C. Remove the bit of stem and dried-up calyx from the base of the fruit; observe, above the calyx, the disk on which the pistil stood. Note with the lens and count the minute whitish, raised knobs at the bottom of the saucer-shaped depression left by the removal of the disk. What are they?

D. Make a transverse section of the lemon, not more than a fifth of the way down from the stigma end, and note:

1. The thick skin, pale yellow near the outside, white within.
2. The more or less wedge-shaped divisions containing the juicy pulp of the fruit. These are the matured locules of the ovary; count these.
3. The thin partition between the cells.
4. The central column or axis of white pithy tissue.
5. The location and attachment of any seeds that may be in the section.

Make a sketch to illustrate these points.

E. Study the section with the lens and note the little spherical reservoirs near the outer part of the skin, which contain the oil of lemon which gives to lemon peel its characteristic smell and taste. With the razor cut a thin slice from the surface of a lemon peel, some distance below the section, and at once examine the freshly cut surface with a lens to see the reservoirs, still containing oil, — which, however, soon evaporates. On the cut surface of the pulp (in the original cross section) note the tubes or sacs in which the juice is contained. These tubes are not cells, but their walls are built of cells.

F. Cut a fresh section across the lemon, about midway of its length, and sketch it, bringing out the same points which were shown in the previous one. The fact that the number of ovary locules in the fruit corresponds with the number of minute knobs in the depression at its base is due to the fact that these knobs mark the points at which fibro-vascular bundles passed from the flower stalk into the cells of the fruit, carrying the sap by which the growth of the latter was maintained.

Note the toughness and thickness of the seed coats. Taste the kernel of the seed.

G. Cut a very thin slice from the surface of the skin, mount in water, and examine with a medium power of the microscope. Sketch the cellular structure shown, and compare it with the sketch of the cork of the potato tuber.

Of what use to the fruit is a corky layer in the skin?

REFERENCE. Strasburger-Hillhouse, 6.

53. A drupe, the cherry. Make a cross section of a partly grown cherry, in which the stone has not become too hard to cut. Make a magnified sketch of it, showing the double *pericarp*, consisting of the *exocarp*, or fleshy part, covered with a thin, tough epidermis, and the *endocarp*, or stone, containing the seed. Crack some ripe cherry stones and study the seeds.

If possible, compare with the structure of the cherry that of other drupes, such as the peach, the fruit of the cocoanut (with the husk), the entire fruit (with husk) of walnut, butternut, or hickory nut, and the fruit of the *Cornus*, or dogwood.

54. An accessory fruit, the strawberry.

A. Study the flower of a strawberry, noting particularly the number, shape, and position of the pistils.

B. Examine a series of strawberry fruits,[1] beginning at the time when the cluster of pistils shows signs of enlarging. How much does each pistil enlarge? What causes the increased size of the fruit?

C. Study a firm, ripe strawberry with the lens, and draw the ripened pistils, called *akenes*.

D. Cut a lengthwise section of the fruit and sketch it.

What is the main difference in proportions between a head of akenes, like that in *Principles*, Fig. 161, and a strawberry? What is the use of the pulpiness of the ripened receptacle?

55. Development of a fruit. Secure a series of as many stages as possible in the development of some convenient fruit, as the common bean, from the newly fertilized pistil to the full-grown pod.[2]

A. Make drawings of the entire fruit, the earlier stages × 4 or × 5, but all the later ones natural size.

B. Cut thin cross sections and lengthwise sections (through the seed) of a series of fruits and sketch them, using a magnification of about 20

[1] Material preserved in alcohol will answer.

[2] Other leguminous fruits, or any moderately large capsules or berries, will answer. Material in preservative fluid suffices for all but the study of the course of absorbed liquids.

diameters for the earliest ones and ten or less for the later ones. Some of the sections may be treated to advantage with potash solution and acetic acid (Sec. 169). Note the changes in size and shape of the seed, in relative development of seed coat and embryo, and in relative bulk of the style, stigma, and ovary wall (pod) compared with the contained seeds, as the latter mature. After treatment with potash, several steps in the development of the embryo can be made out with m.p. Note that when the developing seed is not more than $\frac{1}{4}$ to $\frac{1}{3}$ the length of the mature (dry) seed, its interior is mainly embryo sac, with a rudimentary embryo at one end. Make several drawings to show stages in the process by which the embryo grows until it fills the sac.

C. If fresh material can be had, cut off under water the stalk to which some well-grown pods are attached. Transfer the stalk (without exposing the newly cut surface to the air) into eosin solution and allow it to stand for an hour or more in a warm, sunny place. Cut transverse and longitudinal sections of the pods as soon as they appear well stained along the edges, and slice off thin layers from the flat surface of a pod. Sketch the distribution of the fibro-vascular bundles (recognized by the stain) in all the sections.

Reference. Strasburger-Hillhouse, 6.

Part II

TYPE STUDIES PRECEDED BY THE STUDY OF THE PLANT CELL

THE PLANT CELL, ITS STRUCTURE AND REPRODUCTION

56. The cell structure of the Spirogyra filament (App. 6).*

A. Examine living material. What is its habit of growth, floating or attached? What is its color? How does it feel between the fingers? Note that it is made up of *filaments*, or threads.

B. Mount two or three filaments in water under a cover glass. Examine with l.p. (low power). Are the filaments branched? Do they vary in thickness? Note the cross partitions that divide the filament into parts called *cells*. Draw the outline of a filament under l.p.

C. Study a filament under h.p. (high power). Do the cells vary in length? How much? Select favorable cells and focus on the cross partitions between them to determine their geometrical form. What is the form of the entire cell? Draw a group of two or three cells on a large scale, noting:

1. The transparent *cell walls* bounding the filament and forming the cross partitions.

* To the Instructor: The exercise outlined in Sec. 56 is an excellent one to acquaint the student with the use of the compound microscope and the interpretation of the geometrical form of structures by focusing up and down. If the instrument has not been used before, the student may be made familiar with its parts and their manipulation as described on pages 10–14, under the heading "The Construction and Use of the Compound Microscope."

2. The one or more green *spiral bands* extending around in the interior of the cell just under the cell wall. Focus on the band above and below, following it around the cell.

D. Place a drop of salt solution (5 or 10 per cent) at the side of the cover glass, and draw it under by means of a small piece of filter paper applied against the opposite edge. Note the contraction of a delicate membrane away from the cell wall, so that the former immediately becomes apparent as a continuous membrane inclosing the green band and other contents of the cell. This membrane and its contents comprise the living substance, or *protoplasm*, of the *Spirogyra* cell, and is the living cell or *protoplast;* its structure will be taken up in the next section. The cell wall is composed of *cellulose* which is not protoplasmic in character, being formed by the protoplast and constituting a protective case around it.

57. The structure of the protoplast of Spirogyra.* *

A. Mount a slide of living *Spirogyra* as described in Sec. 56, B, to study the protoplast. Note under h.p.:

1. That each green spiral band, called a *chromatophore*, contains several denser structures termed *pyrenoids.*
2. A globular or elliptical structure, the *nucleus*, near the center of the cell, held in position by delicate protoplasmic strands which radiate outward to the cell walls. The outline of the nucleus will probably be clearer when the material is stained with iodine, as described in B.
3. A delicate lining, or *plasma membrane*, next the cell wall under which the chromatophore lies imbedded in a layer of protoplasm. The plasma membrane was demonstrated when the protoplast was drawn away from the cell wall by the salt solution, as described in Sec. 56, D.
4. That the interior of the protoplast contains no solid or semifluid substance, except possibly some minute granules, and must consequently be either liquid or gas. Which alternative is suggested by the experiment with the salt solution (Sec. 56, D)?

Draw a large figure of a cell showing the cell walls, plasma membrane, chromatophore with pyrenoids, nucleus held in place by the radiating protoplasmic strands, and the large space in the interior of the cell free from protoplasm.

B. Place a drop of iodine solution (Sec. 169) at the side of the cover glass, and draw it under by means of a small piece of filter paper applied against the opposite edge.

1. Note the coloration, or staining, of the protoplasmic structures. The nucleus usually stands out sharply, and should be drawn if it was not clearly seen in the unstained living cell described in A.
2. Draw a portion of the chromatophore showing a pyrenoid under the highest magnification. There will probably be found a circle of dark granules around the pyrenoid. These are *starch grains*, manufactured by the chromatophore in the presence of sunlight, the process being called *photosynthesis.*

C. *Plasmolysis.* The shrinking of the protoplast away from the cell wall when the cell is bathed in a denser solution, as that of salt (described in Sec. 56, D), is called *plasmolysis.* Plasmolysis is accomplished by the withdrawal of water from the interior of the protoplast through the permeable plasma membrane and cell wall when there is a denser solution outside of the cell. Such a movement of water through a permeable membrane is due to *osmosis* (*Principles*, Sec. 48). The plasma membrane of the protoplast is normally held against the cell wall in the living cell by pressure from within, and that condition is called *cell turgor.* The fluid within the protoplast is termed *cell sap* and is contained in cavities called *vacuoles.* The cell sap of *Spirogyra* is in one large vacuole occupying the central region of the cell, in which the nucleus is swung like a hammock by radiating strands of protoplasm. If the facts and principles illustrated by plasmolysis in *Spirogyra* are not clear, repeat the experiment outlined in Sec. 56, D.

D. Place some living *Spirogyra* in alcohol and after several hours note the extraction of a green pigment, *chlorophyll*, from the chromatophores in the filaments. What change in the color of the filaments? The alcohol may be evaporated by gentle heat in a shallow dish, leaving the chlorophyll as a green residue.

58. Photosynthesis in Spirogyra.

A. Perform the experiment in photosynthesis outlined in Exp. XXXI, using *Spirogyra* for the subject.

B. Place *Spirogyra* for a day or two in the dark and then test for starch as described in Sec. 57, B, 2. Return the material to sunlight, and after several hours test again. Compare results.

59. Cell reproduction in Spirogyra. New cells arise in *Spirogyra* either (1) by *cell division* or (2) by *cell unions* to form reproductive cells called *zygospores* or *zygotes*.

60. Cell division in Spirogyra. Search a slide of *Spirogyra* for adjacent cells in the same filament considerably shorter than the average size. Such a pair will probably be sister or daughter cells formed by the division of a mother cell. The division of the mother cell is preceded by the division of the nucleus, after which a partition wall of cellulose is formed between the daughter nuclei. *Spirogyra* is, however, not a favorable subject for the study of nuclear and cell division (Sec. 65).

61. Cell unions to form zygospores in Spirogyra.* * At times *Spirogyra* fruits.[1]

A. If living material is available, note the frequent change in color and occasional dirty appearance of the filaments. Mount fruiting material (either living or preserved) teased out well. Note:

1. That certain cells contain thick-walled oval or elliptical structures densely filled with protoplasm and food material. These are *zygospores* or *zygotes*.
2. That the zygospores are formed by the union or conjugation of cells. In some species of *Spirogyra* the cell unions are between different filaments, in other species between

[1] The terms *fruit* and *fructification* will be used in Part II in an untechnical sense to designate various forms of reproductive organs and processes.

adjacent cells of the same filament. If the conjugation is between different filaments, are the zygospores all formed on one side or are some formed in the cells of one filament and some in the other?

B. Find and draw a number of stages under h.p. illustrating the history of the cell union or conjugation. Note:

1. That the union takes place through processes put out from adjacent cells. These unite to form a connecting tube.
2. That the protoplast from one cell passes into the other and fuses with its protoplast.
3. That the product of this cell union is a fusion protoplast, which forms a heavy wall about itself, thus becoming a well-protected reproductive cell or *spore*. Note the changed appearance of the contents of the spore, and the presence of food material. Test for starch.

Cell unions of this character are sexual processes. The cells which unite are called *gametes* and their product is a sexually formed fusion cell. The fusion cell in *Spirogyra* is called a *zygospore*, or *zygote*, because the gametes are similar. For this reason, also, this type of sexual reproduction is called *isogamy* (meaning similar gametes).

REFERENCE (on the plant cell). *Principles*, Chap. XVIII.

QUESTIONS.* Describe the cell structure of *Spirogyra*. What part of it is living substance and what part of it is non-living? Why are the cross walls in the filament flat planes? What would you expect to be the form of the wall at the free end of a filament? How are new filaments of *Spirogyra* formed? How do the filaments grow and is the growth confined to any special region? What are the essential features in the formation of zygospores which define it as a sexual process? What part does the zygospore play

* TO THE INSTRUCTOR: The sets of questions presented in connection with the type studies of Part II are intended to bring before the student fundamental principles in connection with his laboratory and field work, and his reading. Written or oral exercises may be planned on them if desired.

in the life history of the plant? How is it adapted for its purposes? Construct a series of diagrams that will outline the life history of *Spirogyra*. What are believed to be some of the advantages to an organism in having a method of sexual reproduction?

62. Cell structure of the moss leaf compared with Spirogyra.

A. Mount a moss leaf in water and draw a group of cells under h.p., and show details of protoplasmic structure in one of them. Note:

1. That the chlorophyll is contained in numerous small, disk-shaped bodies called *chloroplasts*. How are they distributed in the cell?
2. The multiplication of the chloroplasts by simple constriction. Draw stages showing their division.

B. Plasmolyze the cells with salt solution, and draw a group. Why do adjacent cells have flat side walls?

C. Stain with iodine.

1. Where are starch grains formed? Draw.
2. Where does the nucleus lie?
3. How much of the cell is filled with cell sap?

63. The Amœba (App. 7).

A. Gather with a pipette some of the slime at the bottom or scum on the top of a culture of *Amœbæ*. Search, under m.p., for transparent, naked cells which slowly change their outline by thrusting out some processes, *pseudopodia*, and withdrawing others.

B. Study under h.p. the changes in form of an *Amœba* as it slowly moves along, making a series of outline sketches. Note the flow of the granular cytoplasm into the pseudopodia as they are formed.

C. Draw diagrammatically an individual on a large scale, showing:

1. The plasma membrane, colorless and without granules.
2. The granular cytoplasm inclosed by the plasma membrane, frequently containing food inclusions, as, for example, one-celled plants such as diatoms and desmids. How would you expect this food to be taken into the interior of the *Amœba?*
3. A dense spherical nucleus (not always easily found).
4. Vacuoles which form, and later suddenly disappear, and consequently are called *contractile vacuoles.*

D. The *Amœba*, as is generally the case with an animal cell, is a naked protoplast. Compare with a typical plant cell. What does one have that is lacking in the other? What do both have in common?

The *Amœba* reproduces by construction, a single individual thus forming two similar daughter *Amœbæ* (see *Principles*, Fig. 167, *B*).

64. Circulation of protoplasm in the cell. Use *Elodea*, or *Nitella*, or stamen hairs of *Tradescantia*.

A. Mount young leaves of *Elodea* in water. Examine the simple cell structure and find a favorable region for detailed study.

1. Note the position and form of the chloroplasts, the nucleus, the cytoplasm, comparing with previous studies on plant cells.
2. Study the circulation of protoplasm next the wall of the cell. Focus on a chloroplast as it moves along, trace its path in a simple sketch or diagram, and determine how long it takes to travel a certain distance measured with the micrometer. Warm the slide gently. What is the effect upon the rate of movement? Describe the movement carefully. Is the direction the same in all cells? Does the substance of the plasma membrane move, or is it granular cytoplasm under the membrane?

B. Mount a portion of the stem of *Nitella*, including uninjured internodal cells. Note the line called the *neutral zone*, free from chloroplasts, which runs diagonally across the cell. The protoplasm on either side of this line moves in opposite directions (see *Principles*, Sec. 230).

C. Cut the stamens out of an opening flower or large bud of *Tradescantia* and mount in water. The stamen hairs are chains of large and very beautiful cells in which the nucleus and arrangement of the cytoplasm may be seen with especial clearness. Are there chloroplasts present? What gives the peculiar reddish violet color to the cell?

Draw a cell on a large scale under h.p. and show the position of the nucleus, and the moving streams of protoplasm, and indicate the directions of the flow by arrows.

65. Nuclear and cell division in the root tip of an onion or other region of growth (App. 8). The details of nuclear structure and nuclear division, called *mitosis*, can only be studied in material carefully killed and hardened to preserve the soft protoplasm as nearly as possible in its normal condition. Thin sections must be cut with an instrument called the microtome and these stained to differentiate the protoplasmic structure (Sec. 211). One of the best stains is a combination of safranin (red) and gentian violet (blue).

A. Examine sections under l.p. to determine the relation of regions or tissues, and diagram the position of the *root cap*, the undifferentiated *embryonic tissue* at the growing point, and the beginnings of the differentiation which appears back of the growing point.

B. Select a typical well-stained cell in the resting condition. Draw under h.p. and note:

1. The *nucleus* with one or more deeply stained globules, each of which is a *nucleolus*, or *nucleole;* a loose network containing *chromatin;* the *nuclear membrane;* the *nuclear cavity* which contains *nuclear sap.*

2. The *cytoplasm*, granular in structure, probably containing one or more *vacuoles* which were filled with *cell sap*.
3. The *cell walls* separating the protoplasts from one another.

C. Find a nucleus in the midst of division (metaphase of mitosis). Note:
1. That the chromatin has become organized into a number of rod-shaped bodies, *chromosomes*, which are grouped in the center of the cell, and that the nuclear membrane has disappeared. Draw.
2. That the chromosomes are arranged in a plate, *equatorial plate*, between the poles of a *spindle* composed of delicate *spindle fibers*. Try to count the chromosomes.
3. Search for evidence of a lengthwise division of the chromosomes into daughter chromosomes. Draw.

D. Find a cell in which the daughter chromosomes have separated into two sets and are passing towards or have been gathered at the poles of the spindle (anaphase of mitosis). Note:
1. The separation of the daughter chromosomes into two groups and their passing to the poles of the spindle to form the *daughter nuclei*. Try to count the chromosomes in each daughter group and compare with the count at the equatorial plate. Draw.
2. The persistence of the spindle between the groups of daughter chromosomes.
3. The appearance of a delicate plate across the spindle, finally reaching the sides of the cell. This is the *cell plate* and marks the position of the new cell wall which will be formed, dividing the *mother cell* into two *daughter cells*, each with a nucleus. Draw.

E. Find a later stage after the daughter nuclei have become organized as resting nuclei and the cell division is completed.

F. Study the beginnings of nuclear division (prophase of mitosis) before the spindle is formed and the chromosomes are gathered at the equatorial plate (metaphase). Note:
1. That the loose chromatin network becomes a thread, *spirem*.
2. That this thread divides transversely into segments, which are the chromosomes. Draw.
3. That the spindle is developed from accumulations of protoplasm (kinoplasm) at two poles outside of the nuclear membrane. Draw.

66. Characteristics of some organic compounds found in plant cells. Certain organic compounds will be met so frequently in cell studies upon the lower plants that some of their characteristics should be known. They fall into two great classes: (1) the *carbohydrates*, composed of carbon, oxygen, and hydrogen, represented

by starch, sugar, cellulose, oils, and fats; and (2) the *proteids*, which contain nitrogen, sulphur, and in some cases phosphorus, in addition to carbon, oxygen, and hydrogen. The principal tests for these substances and some characteristics of their appearance are given in Part I as follows: (1) starch, Sec. 12, A; (2) sugar, Sec. 12, B; (3) cellulose, Sec. 12, C; (4) oils and fats, Sec. 12, E; (5) proteids, Sec. 12, F.

THE FLAGELLATES, OR FLAGELLATA

67. Euglena (App. 9). Study its habits in a glass dish placed near a window. Do the organisms congregate in any part of the dish? Why?

A. Mount in a drop of water and examine under l.p. Describe movements. Under h.p. study cell structure. Note and draw:

1. The naked protoplast; arrangement and form of the chloroplasts.
2. A red pigment spot at the forward end. Can you suggest its possible function with reference to the behavior of the organism towards light?
3. The structure of the forward end with a narrow, slit-like opening leading into a cavity; the position of a long, hair-like *flagellum*, or *cilium*. These structures will probably become clearer after staining with iodine, as described in B.

B. Drain off as much water as possible from under the cover glass and then place a drop of iodine solution at the side. It will slowly diffuse through the water, killing and staining the *Euglenæ*. Watch and describe the effect. Draw details of the forward end, showing the flagellum and the opening.

C. Study *Euglena* in the *encysted* condition, when the protoplast is surrounded by a protective wall. Search for examples of cell division while in this condition. Draw.

THE SLIME MOLDS, OR MYXOMYCETES

68. The spore fruit of a slime mold. The fructifications of the slime molds are certainly plant-like and have been studied and classified chiefly by botanists. Such types as *Stemonitis*, *Arcyria*, *Hemitrichia*, and *Lycogola* are favorable for study.

A. Draw a habit sketch of the spore fruit. Note:

1. The character of the attachment, whether or not stalked; the *spore case*.

2. The *wall* of the spore case. Has it a cellular structure?
3. The thread- or net-like structure, *capillitium*, within the spore case and the powdery spore mass.

B. Under h.p. draw a portion of the capillitium, showing markings, and a group of spores.

69. The plasmodium. This stage in the life history, when available, may be made the subject of very interesting studies on the structure and behavior of protoplasm.

A. Mount a small portion and examine under low and high powers. Note its consistence, structure, and contents. Describe and identify the food contents as far as possible. Is starch present? Are oils or fats present? Do you find any microscopic organisms which have been ingulfed by the plasmodium?

B. Place the plasmodium on moist blotting paper under a bell glass. Devise experiments to determine its reaction:

1. To bright illumination coming from one direction, with darkness or faint illumination on the other side.
2. To warmth on one side.
3. To moisture on one side contrasted with dryness on the other.

C. Should the plasmodium begin to fructify, trace and describe the development of the spore cases.

70. The flagellate-like stage of a slime mold. Try to germinate fresh spores in a hanging drop (Sec. 204) or a covered watch glass. Use water in which decaying wood has been soaked. Study the structure and habits of the motile protoplasts derived from the spores; also the amœboid condition, *myxamœbæ*, which follows, and trace if possible the union of the myxamœbæ to form a new plasmodium.

Reference (to slime molds). Macbride, 38.

THE BLUE-GREEN ALGÆ, OR CYANOPHYCEÆ

71. Field work on the blue-green algæ. Good displays of the blue-green algæ may be found in open drains and stagnant pools which are somewhat foul. Ditches and pools in salt marshes will furnish excellent material. Slimy, dark green growths on the surface of damp flowerpots, woodwork, and earth are frequently composed of these growths. Water blooms are generally made up of either blue-green algæ or *Euglena*.

Make collections in bottles, carefully noting the habitat, and bring to the laboratory for study.

72. Unicellular blue-green algæ. Material of *Glœocapsa* or *Chroöcoccus*, *Clathrocystis* or *Cœlosphærium* is excellent. Study and draw:

1. The form and arrangement of the cells and cell colonies.

2. The structure of cellular envelopes if present.
3. The detailed structure of a cell; the color and its distribution. Are chromatophores present? Can you find a nucleus? How do the cells multiply?

73. Oscillatoria.**

A. Place a small mass of material in a watch glass full of water. What is the color? After a few hours observe the filaments radiating out from the central mass. Explain this habit of growth after the study outlined in B.

B. Mount material well teased out. Under l.p. note:
   1. The filaments. Are they branched or unbranched? Are they of uniform diameter?
   2. The movements of the filaments. Describe and diagram.

C. Under h.p. note and illustrate:
   1. The cell structure at the tip of a filament and the partition walls back of the tip. Compare the length and breadth of the cells. What is their geometrical form?
   2. Draw a group of cells on a large scale, showing the distribution of their granular contents and the coloring matter. (Are chromatophores present? Can you find a nucleus? Where are new partition walls formed?
   3. Note the occasional dead cells. What is the form of the cell wall on adjacent living cells and at the free tips of filaments? Why should the wall take this form? The presence of the dead cells weakens the filament, which breaks apart readily at these points.
   4. How do the cells multiply, and how are new filaments formed? Is cell division confined to any particular region, or is it general throughout the filament?
   5. Search for a very delicate sheath which holds the cells together in a filament, like paper about a roll of coins.

D. Should material of *Lyngbya* be available, it may be studied advantageously at this point in comparison with *Oscillatoria*.

E. Dry a mass of *Oscillatoria* thoroughly, then pulverize and place in a test tube with twice its bulk of water. After

several hours describe the color of the water as seen by transmitted light and by reflected light. This color is due to the pigment characteristic of the blue-green algæ.

74. Nostoc or Anabæna.

A. Study the form and consistency of the colonies of *Nostoc*. Make a habit sketch. Cut out a small portion from a colony and crush under a cover glass. Note under l.p.:

1. The chains of cells, or filaments, imbedded in the almost colorless jelly.
2. Are the filaments branched? continuous?

B. Under h.p. draw part of a filament, showing:

1. The *vegetative cells*, their attachment to one another, method of multiplication, and the character of the protoplasmic contents.
2. The occasional enlarged cells, *heterocysts*, empty or almost empty of cell contents. Two button-like plugs at the ends of the heterocysts, which close what were formerly very small openings into the adjacent vegetative cells. How are the heterocysts distributed throughout the filaments? From what are they developed? Their function is not well known, but the filaments tend to break apart at either side of these cells. How then are new filaments formed?

C. Material of *Anabæna* with *resting cells*, or spores, is more interesting than *Nostoc*, and furnishes an excellent comparative study with that type, or may be substituted for it. Study the general morphology as in *Nostoc*, and especially the structure, position, and development of the resting cells.

Reference (on the blue-green algæ). *Principles*, Secs. 207–211.

Questions. What are some of the life conditions under which the blue-green algæ live? Describe the life history. How may forms without differentiated resting cells, or spores (as *Oscillatoria*), survive unfavorable seasons of drought or winter? In what respects is the cell structure of these plants simpler than that of *Spirogyra*? Compare the morphology of the blue-green algæ with that of the bacteria (if studied).

75. Tolypothrix or Scytonema. These types are especially interesting for the peculiar method of branching, called *false branching*. Study the general morphology of the filament with special reference to the relation of the branches to the heterocysts. Find the beginnings of a branch and note that it breaks through the sheath which incloses the vegetative cells. The heterocysts are more or less firmly united to the sheath, while the vegetative cells may slip along within it. The multiplication and growth of the vegetative cells between the heterocysts, as fixed points, bring pressure to bear which results in the rupture of the sheath and formation of a branch.

76. Glœotrichia. This type should be studied chiefly for the remarkable *resting cells*, or spores, formed next the heterocysts at the bases of the radiating filaments, and for the attenuation of the filaments into long hairs.

THE GREEN ALGÆ, OR CHLOROPHYCEÆ

77. Field work on the green algæ. The green algæ live under a variety of conditions, with several characteristic habitats: (1) there are the growths in clear pools and ponds with floating filamentous masses (pond scums), free-swimming forms (members of the *Volvox* family), attached filamentous or expanded types (*Œdogonium*, *Vaucheria*, *Chætophora*, *Coleochæte*, etc.), and the sediment, rich in desmids, diatoms, and many other one-celled types; (2) there are the growths in slowly running water of streams and on the borders of lakes (frequently *Ulothrix*, *Stigeoclonium*, *Draparnaldia*, *Cladophora*, and the stoneworts); (3) there are the growths just above and below low-water mark on rocks along the seacoast (chiefly sea lettuces, *Ulothrix*, and *Cladophora*); (4) there are the slimy growths on the trunks of trees and stone walls (*Pleurococcus* and other one-celled relatives), and filamentous forms on the earth (*Vaucheria*).

Studies should be made of some of these habitats, collections gathered and examined in the laboratory, and the principal genera identified. Notes should be taken in the field describing the appearance of the algæ as regards size, texture, and color, and their growth habits in relation to light, depth of immersion, and other factors.

78. Pleurococcus.** Gather pieces of green stained bark from the north side of trees, or scrapings from old fences.

A. Note the color and powdery appearance of the growth over the surface and its thickness in places where the growth separates as small scales. Moisten the bark and note the brighter color.

B. Scrape off some of the moistened *Pleurococcus* and mount in water, well teased out. Examine under l.p. to find favorable material. Under h.p. draw:

1. Groups of cells in outline, showing their loose attachment to one another, except just after cell division, when the *daughter cells* are to be found in pairs.
2. A single large cell, showing the *cell wall* and the *protoplast;* a *nucleus* can often be distinguished in the center of the protoplast, and the chlorophyll is generally held in a single large *chromatophore* which may or may not have a *pyrenoid.* These points are brought out more clearly by staining with iodine.

Questions. In what respects is the cell structure of *Pleurococcus* higher than that of the blue-green algæ? What is the life history of *Pleurococcus?* Is it easily killed by winter's cold and summer's drought, judging from its appearance on trees and in other situations? Make a study of the distribution of *Pleurococcus* on a tree trunk, noting the limits of growth and the regions of its greatest luxuriance. Try to determine the reasons for the limits of growth.

79. Sphærella or Volvox (App. 10). These types and others of the *Volvox* family, when available, are especially interesting for their life habits and cell structure, and in the higher types for the complex cell colonies and methods of sexual reproduction.

A. In water swarming with *Sphærella* note the reaction of the organism to light when the vessel is placed near a window.

B. Under h.p. study the movements of the cell and the cell structure, later killing and staining with iodine as described for *Euglena* (Sec. 67, B). Note and draw:

1. The thick, somewhat gelatinous *cell wall.*
2. The protoplast with two *cilia*, red *pigment spot*, and large *chromatophore.* Which is the forward end as the organism swims?

C. Should *gametes* be developed and begin to conjugate, or should the large vegetative cells form thick-walled *resting cells*, these processes may be studied.

D. *Volvox* as an example of a very highly organized cell colony may be compared with *Sphærella* or other one-celled forms of the same family.

Study its swimming habits and its reaction to light in a vessel. Note the points of similarity of its protoplasts to the cells of *Sphærella*. Study the structure of the cell colony and the method of forming daughter colonies. Study the development of the eggs and their change into oöspores after fertilization, and, when material is present, the formation of the packets of sperms. Stained preparations may be studied (Sec. 212).

References (on the *Volvox* family). Goebel, 16, p. 34; Engler and Prantl, 39; *Principles*, Sec. 215.

80. Hydrodictyon, the water net (App. 11). This type, which is common in some regions (as in parts of the East and Middle West), illustrates exceptionally well the features of a cell colony and its methods of reproduction. The cells in older colonies are *cœnocytes*, that is, contain many nuclei. They have a large, irregular *chromatophore* with numerous *pyrenoids*. Permanent preparations in balsam, stained with hæmatoxylin (Sec. 182), show these points well. The pyrenoids are especially favorable for the study of starch formation (see paper of Timberlake, *Annals of Botany*, Vol. XV, p. 619, 1901).

Reference. Goebel, 16, p. 39; *Principles*, Fig. 179.

81. Ulothrix, Draparnaldia, or Stigeoclonium. *Ulothrix* is the best for the study of zoöspore formation, but the other types have a more complex and interesting morphology.

A. Observe the attachment and appearance of the growth.

B. Pick off some filaments and mount in water. Under l.p. study their general morphology. Are they branched or unbranched? Try to find the basal cell of a filament with its attachment, *holdfast*, and compare with the cells in the middle regions and at the ends. Make outline sketches illustrating these points. Is growth confined to the tips, or is it general throughout the filament?

C. Under h.p. study the cell structure. Note:

1. The form of the cells, the band-like *chromatophore* with *pyrenoids*. Draw in detail.
2. Stain with iodine to bring out the *plasma membrane* and *nucleus;* starch grains may be observed around the pyrenoids.

D. Study the *zoöspores* (best observed in the early morning hours). In *Ulothrix* note: ——

1. That the zoöspores are developed in varying numbers in the cells. Make counts and draw the various conditions. Certain striking peculiarities of the zoöspores, the *pigment spots* (See 3), are easily recognized at this time.
2. The escape of the zoöspores from the parent cells, *sporangia*, and their swarming movement in the water. They are sometimes called *swarm spores.*
3. The structure of the zoöspore, showing *pigment spot*, *chromatophore*, number and position of the *cilia*, generally four in number and made clear when material is stained with iodine as described in Sec. 67, B. Which is the forward end of the zoöspore? Draw.
4. Should two-ciliate motile cells be present, they may be expected to unite, or *conjugate*, in pairs in the water, showing that they are sexual cells, or *gametes.* The products of the fusion are four-ciliate cells with two pigment spots. These are *zygospores*, or *zygotes.*

Because the form or morphology of these gametes is similar, this type of sexual reproduction is called *isogamy.*

E. Note that the zoöspores gather on the illuminated side of the vessel and settle down to germinate. After the material has been in the vessel for three or four days, observe the growth of young plants, or *sporelings.* Gather some of the sporelings with a pipette and draw a number of stages illustrating the germination of the zoöspore. Observe the older sporelings, taking on the appearance of the parent plants, and the development from the basal cell of a *holdfast.*

Reference. *Principles*, Sec. 217.

Questions. Describe as fully as possible the life history of *Ulothrix* or whatever other form may be studied. What organisms do the zoöspores resemble? In what particulars? Of the two periods in the life history, the motile and quiescent, which represents the more primitive condition of plant life? Which is now the more important for vegetative activities? Which for reproductive?

82. Ulva, the sea lettuce. Follow in general the outline for *Ulothrix*, noting the different morphology of the plant but similar structure of the individual cells. Study especially the margins of the thallus where zoöspores may be developed.

Reference. *Principles*, Sec. 218.

83. Cladophora. Follow an outline similar to that given for *Ulothrix*, noting the different morphology and very different cell structure. The older cells contain many nuclei, i.e. are *cœnocytes*, and have either a net-like chromatophore with pyrenoids, or numerous somewhat irregular chloroplasts. Permanent preparations in balsam stained with hæmatoxylin (Sec. 182) show these points well.

84. Zoöspores, their formation and habits. Use good material of *Ulothrix*, *Draparnaldia*, *Stigeoclonium*, *Ulva*, or *Cladophora*. Place considerable fresh material in a glass vessel brightly illuminated on one side.

A. Zoöspores may be developed the next day, or perhaps a day or so later. If formed, note:

1. At what time they appear in greatest quantity as a green cloud, and in which part of the vessel.
2. Are they developed in the plant during the daytime? This will require the examination of material at various times of day.
3. Can the time of their escape from the plant be delayed by keeping material in the dark?

B. A full study of the process of zoöspore formation would require the killing and preservation of material at intervals during the night.

85. Œdogonium.**

A. Observe the habit of the plant, the general morphology of the filaments. Are they branched or unbranched? Under h.p. draw:

1. The end of a filament and some cells in the middle region crossed at one end by delicate lines, the *caps*. Note the large *chromatophore* almost filling the cell.
2. The remarkable disk-like *holdfast* developed by the basal cell, especially well shown in younger plants.

B. Study fruiting material under h.p. Draw:

1. The female organ, or *oögonium*, which is a large swollen cell that develops a single female gamete, the *oösphere*, or *egg*. Note the rounding off of the egg before fertilization as a *naked protoplast*, and the formation of a *pore* or *cleft* to allow the entrance of the sperm.
2. The *oöspore* within the oögonium developed from the *fertilized egg*, which forms a heavy wall about itself. Observe the changed appearance of the cell contents, due to the presence of much food material. Test for starch.
3. The male organs, or *antheridia*, groups of small disk-shaped, almost colorless cells, each of which develops two *sperms*. The sperms have a circle of cilia at one end.

Because the form or morphology of these gametes (eggs and sperms) is unlike, this type of sexual reproduction is called *heterogamy* (meaning unlike gametes).

C. The large zoöspores may be present in living material. These are formed singly in the cells. Note their slow swimming and the circle of cilia at one end.

Should the material of *Œdogonium* be of a species with the peculiar dwarf male plants, the laboratory directions would have to be considerably changed.

Reference (on the formation of the caps). Goebel, 16, p. 44.

Questions. What advances does *Œdogonium* show over *Ulothrix* (1) in the structure of the vegetative cells, holdfasts, and tip of filaments? (2) in the sexual organs and gametes? Would the oöspore from its structure be expected to germinate at once, or is it fitted to carry the plant over unfavorable seasons? Describe the life history of *Œdogonium*.

86. Coleochæte (App. 12). If living material is available, study its expanded growth over the substratum, as illustrated by some of the commonest species. Preparations stained in hæmatoxylin (Sec. 212) and mounted entire in balsam are excellent for detailed examination.

A. Under l.p. note the radiate arrangement of the cells from a center of growth. Is the disk one layer of cells thick or more? Where does cell

division and growth take place? May the disk be compared to a system of radiating and branching filaments adhering to one another side by side in a plane? Draw the outlines of several plants of different ages, showing variety of form and appearance of lobes. Draw in detail the cell structure of a sector from the center to the margin.

B. Search for sexual organs, *oögonia* and *antheridia*, near the margins of the plants:

1. The oögonia become large cells, in most forms with a delicate extension like a long-necked flask, and each develops a single *egg*. The tip of the extension opens, allowing the sperms to enter the oögonium.
2. The antheridia are small, almost colorless cells, generally present in small groups near the margin. The *sperms* are two-ciliate.
3. After fertilization the egg forms a heavy wall about itself, thus becoming an *oöspore*. Short filaments then develop from the cell under the oögonium, and these surround the oögonium with a cellular protective envelope, and the entire structure becomes a conspicuous fructification.

87. Desmids. Excellent studies are furnished by species of *Closterium*, *Cosmarium*, *Docidium*, *Micrasterias*, etc., and among the filamentous forms by *Hyalotheca*, *Desmidium*, etc. Make a general examination of sediment from sunlit pools, or material skimmed or strained from the water, for a favorable type.

A. Study the cell structure under h.p. Note and illustrate:

1. The symmetrical halves of the cells with the *nucleus* centrally placed between and a *chromatophore* in each half.
2. The *pyrenoids*, generally conspicuous.
3. In *Closterium* a vacuole near each end containing dancing granules (magnesium sulphate).

B. Material illustrating the conjugation of desmids to form zygospores is not common. Studies may be made from prepared slides. Note:

1. That the *gamete protoplasts* escape from the parent cells, whose halves split apart, and unite outside in the water to form the *zygospore* or *zygote*.
2. That the zygospore has a heavy protective wall elaborately marked in some species.

88. Pond scums.** An outline for the study of *Spirogyra* is given in Secs. 56, 57, 61. Similar studies may be planned for *Zygnema* and *Mougeotia*, which differ from *Spirogyra* and from one another chiefly in the form of the chromatophores and the position of the zygospores between the conjugating cells.

89. Diatoms. Good material is generally present in sediment from sunlit pools such as will furnish desmid material. Brown scums or brown slimy coatings are frequently almost pure growths of diatoms. Make a general examination of material and note especially stalked forms and those united into filaments or chains. Search especially for large, boat-shaped types (generally *Pinnularia* or *Navicula*) that move to and fro in the water. Study the cell structure of these latter forms under h.p. Draw:

A. An *upper* or *valve* view (elliptical in outline) which shows a suture, *raphe*, running almost the entire length of the cell, and a *nodule* at either end and in the middle region. Two brown *chromatophores* lie along either side of the cell and the *nucleus* in the center. Note the globules of *oil* in the cell. Draw an outline on a large scale, showing the markings on the *siliceous shell.*

B. A *side* or *girdle* view (rectangular in outline) which in a large cell will show at the ends an overlapping of the two siliceous shells, inclosing the protoplast, which fit together as a cover fits over a box. Illustrate these points and also the position of the chromatophores, oil globules, and nucleus.

C. Examine some of the polishing powders, such as electro-silicon, for diatoms, making mounts in water or balsam (App. 13).

90. Vaucheria, the green felt.** Some species are terrestrial, growing on damp earth and common in flowerpots in greenhouses. Others are aquatic, forming heavy, dark green mats on the bottom of muddy pools and ditches. Examine the felted growth, its coarseness, feeling, size of filaments, irregular branching. Do the positions of the filaments bear any relation to the direction of light?

A. Carefully mount some filaments in water. Draw under m.p. Is the diameter of the filament the same throughout any considerable length? Are cross walls present? Is the protoplasm uniformly green? Colorless, root-like branches, *rhizoids*, may be found. Under h.p. draw:

1. The end of a filament, showing the arrangement of the protoplasm, the cell wall, the numerous *chloroplasts* and glistening *globules* of *oil*, the position of a central *vacuole* running lengthwise of the filament.
2. Under the highest magnification draw stages in the multiplication of the chloroplasts.

3. Stain with iodine. Is starch present?

4. Material stained with hæmatoxylin and mounted in balsam will show the very numerous minute nuclei. The filaments are therefore *cœnocytes* (see *Principles*, Sec. 229).

B. In fruiting material study and draw the sexual organs, variously grouped in different species.

1. The large oval, female organ, or *oögonium*, sessile on the parent filament but separated from it by a wall. The protoplast becomes a naked *egg* within the oögonium, which forms a *pore* (for the entrance of *sperms*) in a short, beak-like structure somewhat at one side near the tip.
2. The mature *oöspore*, with its heavy wall, developed from the fertilized egg. Note the changed appearance of the contents of the oöspore, now rich in food material.
3. The male organ, or *antheridium*, at the end of a short branch bent like a crook, and separated by a wall from the parent filament. It develops a very large number of minute two-ciliate *sperms*.

Because the form or morphology of these gametes is unlike, this type of sexual reproduction is called *heterogamy*.

C. Allow material to remain in a vessel of water. It may form *zoöspores*.[1] If so, note:

1. Their large size and habits of germination. If studied when motile, observe their slow swimming. Stain the zoöspores with iodine to show the very numerous delicate *cilia* all over the surface; these are really pairs of cilia, each pair above a nucleus (see *Principles*, Fig. 189, *C*). Draw.
2. The formation of the zoöspores at the tips of filaments which appear darker in color. Note the cross wall cutting off a terminal *sporangium*, the protoplasm of which becomes the single zoöspore. Draw.
3. Stages in the germination of the zoöspore and the development of the sporelings.

[1] Material of *Vaucheria* is likely to produce zoöspores in large numbers if placed in fresh water after some days of cultivation in five per cent Knop's solution (Sec. 200, A).

Questions. How would you distinguish aquatic species of *Vaucheria* in the field from *Spirogyra?* from *Œdogonium?* Describe the protoplasmic structure of the filaments. What are the characteristics of a cœnocyte? Do the nuclei occupy fixed positions in the filament? Where in the filament does growth in length take place? Compare the structure of the cœnocytic zoöspore of *Vaucheria* with the protoplasmic contents of a sporangium of such a type as *Cladophora* (see *Principles,* Fig. 184, *B*) or the water molds (see *Principles,* Fig. 214, *C*) and show the points of resemblance. What difference in the behavior of the protoplasm results in the formation of a single zoöspore in *Vaucheria* and of many zoöspores in the other forms? What takes the place of starch in *Vaucheria?* Would the oöspore from its structure be expected to germinate at once? Describe the life history of *Vaucheria.*

91. Chara or Nitella, stoneworts. Examine the growth habits of the plant. Is the surface incrusted with lime? Study the general morphology, noting the well-defined *stems,* the circles of lateral, leaf-like *branchlets,* the joints or *nodes* separated by *internodes,* the growth from a *bud-like tip.* Are these characters which would be expected of a thallophyte? Is the structure a thallus? Sketch these features of the general morphology.

A. In fruiting material, either living or preserved, note the position of the sexual organs, generally in pairs, an *antheridium* and an *oögonium.* If the antheridium is above the oögonium, the form is *Nitella;* if below, *Chara.* Material heavily incrusted with lime may be treated with 1 per cent chrom-acetic acid, which will dissolve it away. Draw a group of sexual organs, showing:

1. For the oögonium, the circle of five spirally wound *filaments,* adhering to one another side by side, which envelop the *egg,* the tips of the filaments projecting beyond the egg as the *crown.* If there are five cells in the crown, the form is *Chara;* if two tiers of five cells each, the form is *Nitella.*
2. For the antheridium the surface view of large, triangular, flattened cells, called *shields* (eight in all), composing the outer envelope.

B. Crush the antheridium, noting the numerous coiled *antheridial filaments,* composed of disk-shaped cells, each of which develops a *sperm.* The attachment of the antheridial filaments by means of a series of

cells to the shields is difficult to determine, and is best omitted in a general study.

C. Crush old oögonia containing thick-walled *oöspores* and note the cell contents full of food material. Test its nature with iodine.

D. Should the material be a species free from lime (especially in the case of *Nitella*) and one whose internodal cell is not covered by corticating filaments, study the circulation of protoplasm in the internodal cell as described in Sec. 64, B.

E. A detailed histological examination of a stonewort is full of interest, but rather special for a general course. Its full understanding demands a study of microtome sections of the growing points, to establish the remarkable method of growth, which can be followed throughout the branchlets and corticating filaments that grow over the internodes in many species. The method of growth furnishes the key to the detailed morphology of the stoneworts.

Reference. Goebel, 16, p. 52.

THE BROWN ALGÆ, OR PHÆOPHYCEÆ

92. Field work on the marine algæ. Study when possible the distribution of marine algæ on rocks at the seaside. Describe the conspicuous growths in three zones: (1) those well above low-tide mark; (2) those at low-tide mark; and (3) those below low-tide mark. Where are the sea lettuces most numerous? the rockweeds? the kelps? the larger red algæ? Examine sunlit tide pools and compare with very shaded pools. If sheltered bays can be studied, note the character of the forms on stones in shallow water, and on the eelgrass. If salt marshes can be examined, study the algal flora along the margins of the tidal streams and in the pools. Make collections of the conspicuous forms, noting their habitats, and bring to the laboratory for examination.

93. Ectocarpus. Examine the habit of the plant, tufted growth, attachment.

A. Draw a portion under m.p. to show the branching. Are the tips of the filaments all alike in structure? Try to determine the method of growth when they end in hairs. Draw a cell under h.p., showing the irregular chromatophore.

B. In fruiting material find either or both of the two forms of sporangia. Draw under h.p:

1. *Unilocular sporangia*, single cells, solitary, sessile, or on short stalks in some species and in chains in others, as in *Pylaiella* (*Ectocarpus*) *littoralis*. Each sporangium develops a large number of two-ciliate *zoöspores*.

2. *Plurilocular sporangia* or *gametangia.* These are branches composed of a very large number of small cubical cells, each of which produces one or perhaps two or three two-ciliate elements similar to zoöspores, but which are known to be *gametes* in some forms, conjugating in pairs as in *Ulothrix.* Trace the development of the plurilocular sporangia from vegetative branches. It should be noted that they are many-celled organs both in structure and origin.
3. Should zoöspores or gametes be discharged from living material, study their movements and then stain with iodine (as described in Sec. 67, B). Note their kidney form and the pair of cilia inserted laterally.

94. Kelps. Study the morphology of such kelps as may be available, noting *holdfast*, *stalk*, and *blade.* Should the blades be in fruit, cut sections in pith to show the one-celled *sporangia.*

95. Fucus, the rockweed.** *Fucus vesiculosus* is perhaps the most convenient species for study. Describe its color, consistency, and life habits if studied living on the rocks.

A. Make a habit sketch of a plant, noting:
   1. Its method of branching, thickened *midrib,* and lateral expanded margin.
   2. The presence of *air bladders* and swollen tips, called *receptacles.*
   3. Sunken regions in the receptacles, termed *conceptacles,* each opening to the exterior by a *pore* through which protrudes a cluster of delicate filaments, *paraphyses.*
   4. The flattened vegetative tips with a *pit* at the end, at the bottom of which lies a group of cells forming the *growing point.*
   5. The disk-shaped *holdfast.*

B. Cut across a receptacle and under l.p. or with a hand lens diagram the distribution of the conceptacles. Note in living material the color of the contents of the conceptacle, whether dark green or orange, and later determine which contain male organs and which female.

C. Dig out with the point of a scalpel the contents of a conceptacle, and mount in water. Study the sexual organs.

Does a plant of *Fucus vesiculosus* have both sexual organs in the same conceptacle? on the same plant? Draw:

1. The female organs, *oögonia*, large cells which develop eight *eggs* each.
2. The male organs, *antheridia*, small cells generally borne in clusters on stalks. Each develops numerous (over one hundred) sperms.

Because of the extreme differentiation of the eggs and sperms the condition in *Fucus* is one of the highest expressions of *heterogamy*.

A plant is called *diœcious* when the sexual organs are borne on different individuals, *monœcious* if both organs are on the same individual.

D. Cut thin sections in pith or study stained microtome sections of receptacles (Sec. 212) and draw on a large scale the outline of a conceptacle, showing the opening with protruding filaments, *paraphyses*, the lining membrane with the sexual organs in position, the network of filaments within the receptacle.

E. Living male and female plants may be separated, rolled up in paper, and kept in a tight box. The contents of ripe conceptacles will then ooze out from the openings. When such contents are placed in sea water the slime dissolves and the gametes, eggs or sperms, are set free. If mixed together the sperms at once swarm around the eggs and the conditions under which fertilization takes place may be observed under the microscope. Note the rotation of the eggs, set in motion by the swarming of the sperms. Sketch the appearance of the eggs. Stain with iodine and draw the sperms under the highest magnification.

F. Fertilized eggs if placed in sea water in a covered watch glass will germinate, developing into sporelings. Draw stages.

Questions. Where do the rockweeds grow? How can they withstand the beating of surf on the rocks? Would the vegetative body of a rockweed be called a thallus? Why? Of what service are the air bladders? Where does fertilization take place in nature. How might the gametes escape from the conceptacles? Why is it not necessary for an egg to become a resting spore (oöspore) in a marine alga? Describe the life history of *Fucus*.

96. Sargassum. Study the general morphology of *Sargassum* from dried specimens on herbarium sheets. Note the *stem*, *leaf-like lateral structures*, branching *receptacles*, stalked berry-like *air bladders*, and *holdfast*. Illustrate these features. Is this structure a thallus?

THE RED ALGÆ, OR RHODOPHYCEÆ

97. Nemalion. Note the form and consistency of the thallus. The color, which may be observed from herbarium material, is not typical of the red algæ.

A. Crush out a tip under the cover glass. Under m.p. study the vegetative structure composed of filaments held together by a gelatinous substance.

1. Diagram the form and arrangement of the filaments in the interior and outer regions.
2. Draw details of the vegetative cells under h.p., showing the peculiar *chromatophores* and the attachment of the protoplasts to one another by delicate strands of protoplasm.

B. From a crushed-out tip of a male, or *antheridial*, plant study the clusters of *sperm mother cells* forming *antheridia* at the tips of the filaments. Draw. Each sperm mother cell develops a single non-motile *sperm*.

C. From a crushed-out tip of a female, or *cystocarpic*, plant study and draw:

1. The large, mature, globular fructification called a *cystocarp*. The development of its spores, *carpospores*, at the tips of filaments.
2. Earlier conditions. Search for the female organ from which the cystocarp is developed. The female organ is a single cell, *carpogonium*, corresponding to an oögonium. It is situated at the end of a short branch, and bears a delicate extension, the *trichogyne*, an organ for the reception of the non-motile *sperms*. Draw a carpogonium with trichogyne, probably having one or more sperms attached, or some early stage in the development of the cystocarp when the fertilized carpogonium may be divided into several cells.

3. Follow the history of the trichogyne through later stages in the development of the cystocarp. Trace the development of the filaments composing the cystocarp.

REFERENCE. *Principles*, Sec. 243.

QUESTIONS. What part of the protoplasm in the female organ corresponds to an egg? Is the structure produced by the fertilized egg a new form of development in your study of the algæ? What is its relation to the sexual plants? Describe the life history and construct a formula expressing the relationships of the different phases (App. 18).

98. Batrachospermum. This fresh-water type of the *Rhodophyceæ* is an excellent substitute for *Nemalion*. Follow a similar outline of study, noting its peculiarities of habit, color, the preliminary growth, *Chantransia*, if present, etc.

99. Polysiphonia. From herbarium specimens examine the general growth habits, method of branching, attachment, and color. Note that there are different forms of plants, characterized by different types of reproductive organs. It is simplest to begin the study with the asexual or tetrasporic plant.

A. The vegetative structure may be studied from any form of the plant. Mount a few filaments. Note under h.p.:

1. That the filaments are composed of rows of cells, called *siphons*, placed end to end and connected with one another by delicate strands of protoplasm; that the cells contain disk-shaped *chromoplasts*. Count the siphons, if possible, by focusing up and down. Draw part of a filament.
2. That the tip of the filament bears clusters of hairs and that it ends in a single cell, the *apical cell*.
3. Crush the filaments or cut sections and note that there is a *central* siphon surrounded by *peripheral* siphons. Diagram their number and arrangement as they would appear in cross section.

B. *The asexual or tetrasporic plant.* Mount filaments bearing *tetraspores*. Draw:

1. Part of a branch under m.p., showing position of tetraspores.
2. A group, or *tetrad*, of four mature *tetraspores* surrounded by the peripheral siphons. Note that the spores are contained in a *mother cell*.
3. Trace if possible the development of the *tetraspore mother cell*, determining its origin from the central siphon and final attachment to the latter through a stalk cell.

C. *The male or antheridial plant.*

1. Sketch in outline the position of the male organs, or *antheridia*, in clusters at the tips of the filaments.
2. Draw the outline of an antheridium on a large scale and fill in the details of a portion showing the outer layer of small, colorless cells which develop the *sperms*.
3. Crush an antheridium and try to determine its general plan of structure. There is a central siphon surrounded by a densely branching system of small cells ending in the sperms at the periphery. Diagram the relation of these parts. Trace the development of the antheridium. It should be clear that the organ is a modified branch.

D. *The female or cystocarpic plant.*

1. The fructification, called a *cystocarp*, is in *Polysiphonia* an urn-shaped structure in which a cluster of *carpospores* is developed from a large cell at the base. Draw a mature cystocarp and afterwards crush out the pear-shaped carpospores.
2. Examine younger cystocarps, tracing the structures back to the female organ, or *procarp*. The procarp is a modified branch and many celled. The carpogonium is enveloped by sterile cells, from among which the *trichogyne* projects somewhat at one side.

The development of the cystocarp is too difficult a subject for general study. It has recently been traced by Yamanouchi (*Botanical Gazette*, Vol. XLII, p. 401, 1906), who has established an alternation of tetrasporic plants with the sexual. The former, together with certain developments from the carpogonium leading to the production of the carpospores, constitute an asexual or *sporophytic* phase in the life history. The sexual plants are *gametophytes* and the *sporophyte* generation begins with the fertilized carpogonium and ends with the formation of the tetraspores, thus including the spore-producing tissues of the cystocarp, together with the tetrasporic plant (see *Principles*, Secs. 245, 246).

THE BACTERIA, OR SCHIZOMYCETES

100. The culture of bacteria on potato * * (App. 14).

A. *Preparation of the culture surface.*

1. Select medium-sized, sound potatoes, and boil with the skins on for fifteen minutes, — not so long that they will not readily hold their form when cut.
2. While the potatoes are cooking, boil six Petri dishes in clear water for fifteen minutes. Lift the Petri dishes out

carefully by the edge, drain off most of the water, but do not wipe. Lay a piece of round filter paper in the bottom of each dish, placing the cover over it at once. It is better that the paper be sterilized by heating in a hot-air chamber at a temperature of 150° C. for half an hour, but this is not necessary if a good quality of filter paper be used. There have now been prepared six moist chambers, *relatively* free from germs. Why?

3. Cut the boiled potatoes into thin slices with a knife that has been thoroughly cleaned and heated in a flame. Place a slice or two on the filter paper in each Petri dish. Be careful not to touch the cut surface with the fingers or any object save the heated knife blade. Lift the cover of the Petri dish carefully, handling only the outer edge, and replace quickly. The culture surface is now ready for inoculation. The boiling of the starch prepares a much better culture medium than the surface of a raw potato.

B. *Inoculation of the culture surface.* Care should be taken to lift off the covers of Petri dishes gently, and replace at once after inoculation.

1, 2. Lift the covers from two dishes and expose the potato to the air for five minutes where it is likely to gather particles of dust. Place one dish in a cool situation (as an ice box), and the other in a warm one (as near a radiator), noting the temperatures with a thermometer.

3. Draw the finger nail twice across the cut surface of a potato in another dish, in two parallel lines half an inch apart.

4. Wash the edge of a public drinking cup or the outlet of a faucet with a cupful of distilled or sterilized water. Place a drop of the water on the surface of the fourth slice, noting its position.

5. Spread a very small quantity of milk over the surface of another potato with the sterilized point of a knife.

6. Leave the sixth potato slice untouched for comparison with the inoculated ones.

C. Label each potato culture with a number and write an account of each, giving (1) day and hour of inoculation, and (2) method. Examine the cultures for several successive days and record the changes from day to day. Describe and sketch the appearance of the growths, their color, form, and consistency.

101. Fluid cultures of bacteria.**

A. *Hay infusion.* Place some hay in a small quantity of water and leave in a warm place for a few days. The scum that forms is principally bacterial and composed largely of *Bacillus subtilis.* Later quantities of infusoria, chiefly *Paramœcium,* will develop.

B. *Mother of vinegar.* Examine the brownish mass called *mother of vinegar* from a vinegar jar or bottle. This mass is a good illustration of what is called a *zoöglœa,* — that is, a gelatinous growth developed by bacteria.

C. *Decaying algœ.* Allow algæ, such as *Vaucheria* or *Spirogyra,* to decay in a bottle in a warm place. A bacterial scum will form that generally contains quantities of the spiral filamentous type, *Spirochæte. Spirochæte* will also generally develop in filtered bean or pea broth made by boiling crushed beans or peas. Note the putrid odor.

102. Microscopical studies of bacteria.

A. Examine under h.p. the scum from a hay infusion (Sec. 101, A) for the hay bacillus, *Bacillus subtilis.* Note:

1. Colorless rod-shaped cells, solitary or forming filaments of various lengths, their movement in the water, and their size as compared with such infusoria as may be present, particularly *Paramœcium.* Draw.
2. Study under very high magnification, if desired, the crosswise division, or *fission,* of the rods. This study will demand an immersion lens.
3. Place some of the scum in distilled water and after several days, when the food supply has become exhausted, study under an immersion lens the small, thick-walled *spores* formed within the rods.
4. Other bacteria will probably be present besides the hay bacillus. Search for spherical types, *Micrococcus,* and filamentous forms, the latter sometimes spirally twisted and motile, *Spirillum* or *Spirochæte.*

B. Mount a small quantity of the "fur" from the teeth spread out in a drop of water. A number of forms will be found (Strasburger-Hillhouse, Fig. 102).

C. Study preparations from some of the brightly colored colonies (red, yellow, pink, etc.) which may develop on the potato cultures, generally composed of very minute forms.

D. Examine mother of vinegar (Sec. 101, B) for small rod-shaped types (*Bacillus aceti*, with other forms).

E. Study the growths from the infusion of decaying algæ (Sec. 101, C) for long, spirally twisted filaments of *Spirochæte* in very active movement.

103. Staining of bacteria. Stained preparations of bacteria frequently show much more clearly than the living forms the structure of the cells and processes of spore formation. The demonstration of cilia requires very high powers and special methods.

A. Mix a minute quantity of bacterial slime (*Bacillus subtilis* is a good form) in a watch glass of water. Spread a film of the water on a cover glass and allow it to dry. The process may be hastened by holding the cover glass, film side up, well above a flame; the heat helps to attach the bacteria to the cover glass.

B. Dip the cover glass in a strong water solution of fuchsin or of gentian violet. Rinse off the stain in water and examine to see if the bacteria are overstained. If so, extract the stain with alchohol. Finally allow the cover glass to dry and mount in Canada balsam.

QUESTIONS. What types of the algæ do the bacteria most resemble in their cell structure and morphology? How do the bacteria obtain their food and what is its character? What changes have you observed produced by growths of bacteria?

THE YEASTS, OR SACCHAROMYCETES

104. The culture of yeast.** Prepare a pint of a five or ten per cent solution of molasses in water.

A. Place a small quantity of the solution in a test tube and add a fragment of fresh yeast. The scum, or sediment, after twenty-four hours will give excellent material of growing yeast for microscopical study.

B. Shake half a yeast cake in a pint of molasses solution and place in a bottle inverted over a dish of water. Note the fermentation indicated by the formation for several days of bubbles of gas, which will collect in the upper region of the inverted bottle. Test the nature of this gas by the aid of limewater in the manner described in Exp. IV. What is the gas? Note the decided odor of *alcohol* in the fluid after fermentation has ended.

C. If convenient distill off about a quarter of the fermented liquid, add a little quicklime to the distillate, and redistill into a carefully cooled receiver. Pour some of the second distillate into a saucer, note its odor, and try to light it.

105. Structure of the yeast cell.**

A. Mount in water some of the scum, or sediment, present in the culture described in Sec. 104, A. Brewer's yeast, if obtainable, is perhaps the best form for microscopical study, especially the "top yeast." Observe the colorless oval cells frequently occurring in short chains or small groups. These are yeast cells. Make drawings to show:

1. A large cell with granular protoplasm containing one or more vacuoles.
2. The formation of new cells by the process of *budding*. Draw stages in the development and growth of the buds, and the formation of chains or clusters of cells.

B. Mount a bit of yeast cake in water. Stain with iodine.

1. What are the large grains composing the bulk of the yeast cake?
2. What is the staining reaction of the yeast cell with iodine? Is there starch in the cell?

Questions. What are the life habits of the yeast plant? What is its food and how do the cells obtain it? Can the yeast cell absorb solid food like an Amœba? Why? What happens during the process of fermentation? What part does yeast play in the raising of bread? What substitutes for yeast may be employed in bread making, and why?

THE ALGA-LIKE FUNGI, OR PHYCOMYCETES

106. Rhizopus nigricans (Mucor stolonifer), the bread mold * * (App. 15). A culture is easily obtained by placing a slice of bread on a rack, or other support, inside a bell jar set in a dish of water to form a moist chamber.

A. Note the gradual development of mold over the substratum (bread), and the color and size of the filaments, or *hyphæ*, which together constitute the *mycelium*. Is the mycelium all above the surface of the bread? Observe the development of upright stalks, in groups, and the black sporangia formed at their ends. Is the direction of the growth of the hyphæ above the surface of the bread influenced by light?

B. Lift off carefully some of the mycelium and mount in water. Note under m.p. the color and structure of a hypha. Is it septate? What alga does it resemble in cell structure except for the absence of chloroplasts? Draw under h.p. a tip showing the cell wall and distribution of the protoplasm. Observe the glistening globules of oil or fat, and watch for streaming movements. Material stained with hæmatoxylin (Sec. 182) will demonstrate the very numerous minute nuclei. Is the filament a cœnocyte?

C. Mount portions of the mycelium with groups of stalks bearing sporangia (sporangiophores). Draw a group of stalks about five times its natural size. Note and draw under m.p.:

1. The base of a group, showing a cluster of root-like filaments, *rhizoids*, that penetrate the substratum.
2. Stages in the development of the *sporangium*, showing (*a*) the enlargement of the end of the filament before the formation of the dome-shaped *columella*, which cuts off the terminal sporangium; (*b*) a sporangium containing developing spores, and showing the position of the columella.
3. The end of a stalk (sporangiophore) after the rupturing of the sporangium wall, exposing the columella.
4. A group of *spores* under h.p.

D. The molds have a method of sexual reproduction, but *Rhizopus nigricans* only exhibits it if certain strains happen to be growing together, and this seems to occur rarely in nature (see Blakeslee, *Science*, Vol. XIX, p. 864, 1904; and *Proc. Amer. Acad. Arts and Science*, Vol. XL, pp. 205–319, 1904). *Sporodinia* (Sec. 107), however, readily develops zygospores. Preparations may be used for this study (Sec. 212).

Questions. What is the food of the mold? How does it obtain its food? Can solid material pass through the wall of the filament? How does it happen that *Rhizopus* springs up so readily on bread? Compare the structure of *Rhizopus* with *Vaucheria*, noting points of similarity and difference.

107. Sporodinia. This form grows on decaying toadstools and mushrooms and frequently forms zygospores together with the sporangia. The spores will grow readily on bread and other media.

A. Study zygospores and their formation from living or preserved material. Draw:

1. A mature *zygospore* situated between two filaments called *suspensors*. Compare their walls. Crush the zygospore and note the dense contents full of oily food material.
2. Stages in the formation of the zygospore showing (*a*) the union of the tips of two filaments, (*b*) the cutting off of terminal cells or gametes which may be called *cœnogametes* since they are multinucleate, (*c*) the fusion of the cœnogametes to form the zygospores.

B. The sporangia of *Sporodinia* make an interesting study in comparison with those of *Rhizopus*.

108. Saprolegnia or Achlya, water molds. Place a dead fly, or a pellet of meat, or bits of bread in a dish containing pond or ditch water. After two or three days note the gradual development of a halo of radiating hyphæ. Make a habit sketch of the growth upon the substratum.

A. When some of the ends of the hyphæ become swollen and white cut out a portion of the mycelium with the scissors and mount in water. Study the hyphæ. What is their structure compared with that of the molds? Draw a tip showing arrangement and character of the protoplasm.

B. Examine the swollen tips, some of which should be *sporangia* with *zoöspores* in various stages of development. Draw under h.p.:

1. A mature sporangium full of zoöspores.
2. Stages in the development of sporangia.

3. Watch for the escape of zoöspores, which almost always takes place from mature sporangia, when mounted on the slide, probably because of the somewhat changed conditions of temperature and density of the water. Observe whether the zoöspores swim away at once (generally *Saprolegnia*) or immediately come to rest near the opening of the sporangium (generally *Achlya*).
4. Stain the zoöspores with iodine to show cilia.

C. Watch for the development of *oögonia* as clusters of minute spherical structures, generally situated nearer the substratum than the sporangia. Observe:

1. That the *oögonium* develops a number of *eggs*. Count them. Draw.
2. Whether delicate *antheridial filaments* are present, growing over the surface of the oögonia and entering them. Draw, if present, and study their origin.

109. Albugo, the blister blight. This parasite is common on the shepherd's purse (*Capsella*), which is the most convenient host for laboratory study. Note the appearance of white blisters, the conidial fructification, on leaves and stems. The sexual fructification, more common on the radish, occurs in the interior of stems and leaves, which become swollen and purplish in color. Make a habit sketch of the blisters.

A. Study sections of the blisters cut free-hand in pith or preparations cut on the microtome (Sec. 212). Draw:

1. An outline of the section showing (*a*) the position of the epidermis, (*b*) the chains of air spores, or *conidia*, which raise the epidermis from the tissue beneath, and (*c*) the position of the *conidiophores*, structures from which the conidia develop.
2. Under h.p. the details of a group of conidiophores, showing the manner in which the conidia develop and the relation of the conidiophores to the *mycelium* in the interior of the host.
3. Study the mycelium between the cells of the host. Search for sucker-like processes, *haustoria*, penetrating the host cells. A portion of infected tissue boiled for a few minutes in a dilute potash solution (five per cent) and then teased out will give excellent preparations.

B. Study sections of tissue with the sexual organs. Note and draw:

1. The large *oögonia*, the oldest ones containing each a single *oöspore* with a heavy cell wall.
2. Younger oögonia in which the egg is present as a region of denser *oöplasm*, separated from a surrounding *periplasm;* also younger stages before the egg is differentiated in the oögonium.
3. Favorable sections, if present, showing the club-shaped *antheridium* at the side of the oögonium and the beak-like process which it puts forth, penetrating the oögonium and growing through the periplasm to the egg.

THE SAC FUNGI, OR ASCOMYCETES

110. Field work on the sac fungi and basidia fungi. The groups of higher fungi, *Ascomycetes* and *Basidiomycetes*, may readily be studied together in the field, since representatives of both are usually found in the same situations. There are several sorts of localities which furnish abundant supplies. (1) Wet, shaded woods and the borders of shaded swamps will give the larger forms of cup fungi and their relatives, and certain basidia fungi. (2) Open woods, especially along woodland paths, are excellent situations for the fleshy gill, pore, and tooth fungi, while stumps, logs, and fallen branches furnish material of the woody basidia fungi and the knot and wart fungi. (3) Pastures and the edges of woodland are favorable situations for certain gill fungi and puffballs. (4) Lichens grow under a great variety of conditions, from those of bare soil and rocks to much shaded situations on tree trunks. (5) The parasitic forms have for the most part their own peculiar life habits associated with various hosts. Collections of conspicuous forms should be made with careful notes on the life conditions, and the forms brought to the laboratory for identification, at least so far as the chief groups are concerned. The woody and firm fungi (including lichens) and many parasitic forms on leaves may be dried or pressed as one would any plant. Fleshy fungi must be preserved in strong alcohol.

111. Microsphæra, the lilac mildew. Study its habit of growth on the lilac. Where is it most luxuriant? Describe the appearance of the mycelium on the leaves. Try to find leaves that appear powdery because of the conidial fructification, commonest in the summer time. Note the position of the *ascocarps* or *sac fruits*, appearing as black dots.

A. *The ascocarps.* Sketch the outline of a leaf and show the distribution of the mycelium with its *ascocarps.* Moisten the surface with a dilute potash solution, and with a scalpel scrape off some of the mycelium and sac fruits.

1. Note the character of the *hyphæ*, their branching, the occasional cross partitions, and the cell contents.
2. Draw an ascocarp with one or more of its *appendages* in detail. What is the structure of the wall of the fruit and the tips of the appendages? Note the number, form, and distribution of the latter.

3. Crush the ascocarps carefully by pressure on the cover glass with the tip of a scalpel while watching them under the microscope. Observe the escape of the sacs, called *asci*, containing *spores*. Note their number and form.
4. Draw an ascus, or group of asci with the spores, *ascospores*. What is the number of spores, their form and arrangement? Show these points in your drawings. The sexual organs of the mildews are small and not easily studied (see *Principles*, Fig. 219, and the paper by Harper, "Sexual Reproduction and the Organization of the Nucleus in Certain Mildews," Carnegie Institution of Washington, Publication No. 37, 1905).

B. *The conidial fruit.* Moisten with a potash solution the surface of a leaf which appears powdery and scrape off the mycelium. Observe the upright filaments, or *conidiophores*, which develop terminally a chain of air spores, or *conidia*. Other types of the mildews are frequently better for the study of the conidial fructification, as, for example, *Erysiphe*.

Questions. Is there mycelium of the mildew in the interior of the host? How does it obtain its nourishment? Which forms of spores, conidia or ascospores, might serve better to carry the fungus over unfavorable seasons, and which to multiply the plants during the growing season? Describe the life history of the lilac mildew.

112. Penicillium and Aspergillus, the green and yellow mildews. The green mildew, or "mold," *Penicillium*, is a very common form and begins to appear on bread shortly after the bread mold has reached a luxuriant development. On what other substances have you observed the green mildew growing? The yellow mildew, *Aspergillus*, may be obtained on cheese in a moist chamber and is not uncommon on damp leather, herbarium material, and other substances that "mildew." The fructifications of these two forms are generally conidial. Describe their appearance.

A. *Conidial fructifications.* Place a small quantity of the fructification on a slide in a drop of alcohol (to drive out air bubbles), followed by water. Distribute the material well and mount. Study under the highest magnification:

1. The spore-bearing stalks (conidiophores), with a portion of the mycelium. Note the cross walls and the arrangement of the *conidia* and their formation. Draw in detail.
2. Diagram and compare the fructifications of *Penicillium* and of *Aspergillus*.

B. Some species of *Aspergillus* not infrequently develop ascocarps (formerly included in the genus *Eurotium*). Study their structure in comparison with the sac fruit of the lilac mildew.

113. Peziza, Lachnea, or other cup fungi.

A. Study the general form of the cups, which are sac fruits or ascocarps, and their relation to the substratum. Where is the vegetative mycelium of the fungus? Tap the cup of large forms, if living, and note the discharge of a smoky cloud of spores. Make habit sketches.

B. Section the cup in pith with a razor, or study sections cut with the microtome (Sec. 212). Draw first an outline sketch showing the relation of the parts, and then details. Note:

1. The fruiting surface containing the spore sacs, *asci*, in various stages of development among sterile filaments, *paraphyses*.
2. The relation of the fruiting surface to the dense web of interwoven hyphæ beneath.
3. Study stages in the development of the asci and ascospores.

C. Some of the larger fleshy *Ascomycetes* related to the cup fungi are excellent for comparative studies, or substitutes. Such are the morel (*Morchella*), *Helvella*, *Geoglossum*, *Mitrula*, etc.

114. Knot and wart fungi. Material of the black knot (*Plowrightia*) and the larger wart fungi, such as *Daldinia*, *Hypoxylon*, *Xylaria*, etc., furnish excellent studies in comparison with the cup fungi.

A. Study the growth habits of the forms.

B. Section with an old razor the sac fruits, *ascocarps*, noting that the sacs are contained in special cavities, *perithecia*, opening to the exterior.

115. Other sac fungi. Many other sac fungi are interesting types for study if material and time are available. Among them are ergot (*Clavíceps*), caterpillar fungi (*Cordyceps*), truffles, some of the spot fungi and rots. Certain of these sac fungi have remarkable forms of conidial fructifications well worth study, as have also many of the imperfect fungi.

116. Physcia, Parmelia, or other lichen** (App. 16).

A. Study the form of the lichen, whether closely pressed against the substratum (crustaceous), lobed and leaf-like (foliose), or much branched (fruticose). Describe its substratum, attachment, color, and form. Search for fruiting

regions, frequently in the form of cups, called *apothecia*. Draw habit sketches.

B. Section in pith with a razor a medium-sized fruit (first moistened). Draw first an outline sketch, showing the relation of parts, and then details. Note:

1. That there are two elements in the lichen: (*a*) green or blue-green cells; (*b*) colorless regions made up of filaments more or less densely interwoven.
2. Study in detail the green or blue-green cells under h.p., comparing their structure with the algal types that have been studied.
3. Examine the colorless regions, comparing their structure with such types of fungi as have been studied.
4. Study in detail the fruiting surface. Is it algal or fungal in character, and to what types is it clearly related? Draw carefully its structure.

C. Study from sections the strictly vegetative parts of the lichen with reference to the distribution of the parts composing it, and especially the structure of the lower surface where it comes in contact with the substratum.

D. Search for scales, called *soredia*, on the surface of some lichens. These scales contain algal cells inclosed in a network of hyphæ, and falling off the parent plant may develop new lichens, thus constituting a very effective means of vegetative propagation.

Reference. *Principles*, Sec. 271; Schneider, 42.

Questions. What are your conclusions on the structure and composition of a lichen? What are its methods of reproduction? How do new lichens develop? Describe the life habits of lichens and their effect on such substrata as rocks, trees. Under what conditions do they grow most vigorously. How do they survive periods of drought and cold? What is the food of a lichen and how is it obtained? How do the algæ benefit the fungus in the lichen? Does the alga receive equivalent benefit from the fungus?

THE BASIDIA FUNGI, OR BASIDIOMYCETES

117. The smuts. The corn smut, *Ustilago Maydis*, is excellent for demonstration, but some of the smaller forms found on oats, wheat, many grasses, and *Juncus* are frequently more convenient for laboratory work.

A. Study the fructification on the host. Observe the parts infected and the extent of the injury. Make habit sketches.

B. Tease out part of the fructification in water. Draw the resting cells known as *chlamydospores* or *teleutospores*. Does their structure indicate that they are resting spores capable of carrying the fungus over unfavorable seasons of drought or cold? Why? Sections of the infected tissue just before the formation of the chlamydospores are necessary to an understanding of their development.

C. Place some of the chlamydospores in a dilute decoction of manure, previously sterilized by boiling, or make a culture in a hanging drop (Sec. 204). The spores germinate better after being frozen. Observe the cultures from day to day and trace the germination of the spores and the development from each of a short filament, *promycelium*, which gives rise to thin-walled, delicate *sporidia*, or spring spores.

118. Puccinia graminis, the wheat rust. It is somewhat easier to begin the study with the stage known as black rust.

A. *The black rust.* Study the distribution of the black rust on the stems and leaves of wheat or various grasses. Make habit sketches. At what time does the black rust appear on the wheat?

1. Scrape out the contents of a spot and examine in water under h.p. Note the color of the resting cells, which are really *chlamydospores*, although generally called *teleutospores*, or winter spores. These spores are always two-celled. Draw in detail.

2. Section the leaves or stem in pith (soaking dried material first in potash solution), or study sections cut with a microtome (Sec. 212). Note the clusters of teleutospores, each on a stalk, and the relation of the latter to the mycelium within the host. Examine the distortion (hypertrophy) of the host tissue in the infected region. Show these points in a large figure or series of figures.

B. *The red rust.* Study the distribution of the spots of red rust. Habit sketch. At what time does the red rust appear

on the wheat? When is it present in greatest quantity? What effect does it have on the host?

1. Scrape out the contents of a spot and examine under h.p. Note the color and form of the spores, called *uredospores*, or summer spores. These spores are always one-celled. Draw.

2. Study sections of the leaves (Sec. 212), as for the teleutospores, and note the origin of the uredospores and their relation to the mycelium of the host. Show these points in a large figure.

C. *The cluster cups on barberry.* Examine the infected barberry leaves, making a habit sketch of the lower side. Draw the spots under a hand lens, noting (1) on the lower side relatively large cluster cups, or *æcidia*, containing *æcidiospores;* (2) on the upper side minute crater-like openings marking the *spermogonia.*

1. Scrape out the contents of a cluster cup and draw the æcidiospores under h.p.

2. Section the spots in pith or study sections cut with a microtome (Sec. 212). Note (*a*) the formation of the æcidiospores in chains from a fruiting surface; (*b*) that the wall of the cluster cup, *peridium*, is a cell membrane composed of rows of modified spores adhering together; (*c*) the small spermogonia filled with very delicate converging filaments which develop terminally bacterioid bodies, believed to be degenerate sperms and called *spermatia*; (*d*) the general relation of these parts to the tissue of the host. Show these points in a large figure or series of figures.

REFERENCE. *Principles,* Sec. 275.

QUESTIONS. Why are the teleutospores thick walled? What do they develop on germination (see *Principles,* Fig. 231)? Outline carefully the life history of the wheat rust from your laboratory study and reading. Compare this life history with that of the smut, noting the phases that correspond with one another. Does the barberry grow in your section of the country, and if so does it produce cluster cups? If it does not grow there how may the life history of the rust be modified?

119. Pore and tooth fungi. Study representatives of the pore or tooth fungi in the field. Note whether they are *parasites* or *saprophytes.*

A. Observe their form, attachment, and relation to the substratum. Examine some of the bracket types with reference to their position in relation to the surface of the earth. What must be the determining influence in shaping their manner of growth? Make habit sketches. These structures are fructifications. Where is the vegetative mycelium?

B. Note the fruiting surface, or *hymenium*, lining cylindrical pores in the pore fungi (Fam. *Polyporaceæ*) and distributed over teeth in the tooth fungi (Fam. *Hydnaceæ*). Cut into the fructifications to ascertain the structure and position of the pores or teeth. Draw in detail.

120. Agaricus, Coprinus, Amanita, or other gill fungus * * (App. 17). Study the type when possible in the field. Is it a *saprophyte* or a *parasite?* The toadstool is a fructification. Where is the vegetative mycelium? Dig up the earth around the base of the toadstool and wash carefully. Examine the white strands of the mycelium under the microscope. Are they single hyphæ?

A. *The fructification.* Note the three parts always present in a toadstool: (1) the *stalk*, or *stipe;* (2) the *cap*, or *pileus;* (3) the *gills*, or *lamellæ*, on the under side of the cap. In addition to these parts there are present in certain genera (4) either a *cup* or *volva*, or both, from the interior of which the stalk rises, and (5) a *ring* around the stalk just beneath the cap. The ring is the remains of a *veil*, present at a certain stage of development, connecting the margin of the cap with the stalk. Portions of the volva are found in some forms as scales on the top of the cap. Show these structures in a habit sketch.

1. Examine in some detail the position of the gills on the cap, their color and texture, and also that of the stalk. Divide the toadstool lengthwise to determine these points.

2. Section the gills in pith or study sections cut with a microtome (Sec. 212). Note the structure of the fruiting surface, or *hymenium*, containing *basidia*, each of which develops four spores, *basidiospores*, on short stalks, *sterigmata.* The common mushroom of the market, *Agaricus campestris*, develops only two spores on each basidium. Observe the relation of the basidia to a network of hyphæ in the

interior of the gill and note their position among the sterile cells in the hymenium. Show these points in a detailed drawing.

3. Observe the color of the spores, best determined by spore prints obtained when the pileus is cut off the stalk and placed gill side down on a sheet of paper and left over night under a bell jar or tumbler. Use black paper if the gills are white.

B. *Development of the fructification.* Study the young "button" stages of the toadstool. Cut them lengthwise and determine the position of the parts of the mature fructification. This examination should make clear the relation of the stalk to the cap above and the cup below, if present. Note that the gills are developed in a chamber whose roof is the cap and whose floor is the veil, finally ruptured by the expansion of the cap and remaining as the ring in some types. Illustrate these points in outline sketches or diagrams.

Questions. What seasonal conditions are best for gill fungi, and what for fleshy, pore, and tooth fungi? Describe the habits of growth of gill fungi in pastures and lawns. What is a fairy ring? Can you account for its form? Do you know of any parasitic gill fungi attacking trees?

121. Puffballs, earth stars, and nest fungi. Study types in the field in reference to the substratum. Where are the vegetative portions of the plant? Examine the fructifications in various stages of development. Make habit sketches.

1. Cut sections lengthwise and observe the texture of the envelopes inclosing the spore chamber. Note the way in which the chamber opens and the spores are distributed.
2. In the spore chamber observe the fibrous remains of sterile tissue and the powdery spores. The nest fungi exhibit special complexities in the form of egg-like structures which contain the spores.

THE LIVERWORTS, OR HEPATICÆ

122. Field work on the liverworts and mosses. The liverworts and mosses can be advantageously studied together in the field since many species of both groups grow in similar situations. The chief types of localities are (1) wet swamps, especially *Sphagnum* bogs, frequented by other mosses and

certain thalloid liverworts; (2) wet margins of ponds and swamps and wet meadows, sometimes supplying *Marchantia* abundantly and many mosses; (3) the surface of small ponds and the mud around their borders occasionally abounding in *Riccia* and *Ricciocarpus*; (4) wet rocks along shaded streams, walls of gorges and entrances to caverns frequently with very extensive growths of liverworts, *Marchantia*, *Conocephalus*, *Pellia*, etc.; (5) base and sides of trees in damp, shaded woods, rocks and the ground in similar situations covered with mats of leafy liverworts and mosses, often mixed together in confusion, but some types presenting characteristic habits of growth; (6) dry earth in more open woods, pastures, bare hillsides, etc., with a number of characteristic types of mosses but no liverworts. Notes should be taken on the life habits, especially with reference to the texture of the forms in relation to the conditions of moisture. Collections may be made and brought to the laboratory for study. For city classes, abundant growths of *Lunularia* and *Marchantia* may frequently be found in greenhouses, especially those not well kept, together with many mosses and their protonemata.

123. Ricciocarpus. Most species of *Riccia* may also be studied by this outline. If living material is available, observe carefully the life habits.

A. *General morphology.* Note:

1. The upper and lower surfaces of the thallus. How are they distinguished?
2. The form of the plant, method of branching.
3. The notched tips or forward ends of the branches where are situated the *growing points*. How do the plants reproduce vegetatively?
4. A shallow furrow, thickened somewhat on the lower side like a *midrib*, running into each lobe, forking when the thallus forks, and ending in the growing points.
5. The position of globular bodies, the older ones black, imbedded in the denser tissue along the furrow. These are *sporophytes*, frequently called the *fruit* of the liverwort. What is the relative arrangement of the younger and older sporophytes?
6. Examine the structure and distribution of membranous *fringes* and delicate filaments, *rhizoids*, on the lower surface. Cut off some of these and examine under m.p. What are their functions? Draw.

Draw a habit sketch of the upper surface, showing points discussed above.

B. *Structure of the thallus.* Cut sections in pith across the thallus and through the sporophytes when possible. Note:

1. The cell structure of the upper and lower surfaces as compared with one another. Where are the chloroplasts chiefly found?
2. The position and attachment of the fringes and rhizoids.

C. *Structure of the sporophyte.* Study sections of the sporopyhtes with developing or mature spores. Note:

1. That the sporophyte is contained within a cellular envelope, *calyptra*. Observe the positions of the sporophytes with relation to the midrib region of the thallus.
2. That the sporophyte has a delicate wall composed of a layer of cells within the calyptra, and contains developing or mature spores.
3. That the spores are formed in groups of four, *tetrads*, within *spore mother cells*.
4. The markings on the walls of mature spores.
5. In median sections the shriveled remains of the *neck* of the *archegonium*, whose very much enlarged base is now the calyptra.

Illustrate as many of these points as possible in a semidiagrammatic drawing.

D. *The structure of the sexual organs and development of the sporophyte.* These are best studied from lengthwise sections cut in paraffin with the microtome and stained.

Reference. Campbell, 23.

124. Marchantia. It is well to examine first, in comparison with one another, male and female plants and those devoted chiefly or exclusively to bud formation. In living material study the growth habits in relation to earth, dampness, and light.

A. *General morphology.* Note:

1. The upper and lower surfaces. How are they distinguished?
2. The ribbon-like form of the thallus, the method of branching. Is it a mode of forking, and why are the branches irregular in length?
3. The notched tips of the branches where the *growing points* are situated. Find a specimen whose tip has recently branched so that there are two growing points close together but diverging.
4. A central line, or *midrib*, running into each branch, forking when the thallus forks, and ending in the growing points.
5. The character of membranous *fringes* and filaments, *rhizoids*, on the lower surface, and their distribution with especial reference to the midrib region.
6. The presence of *stalked disks* or *umbrella-like structures*, which bear the sexual organs, or of *cups*, containing *buds*.

Illustrate as many of these points as possible in sketches.

B. *Structure of the thallus.*

1. Examine the upper surface with a hand lens. Draw a portion showing the diamond-shaped *areas*, each with a central *pore*.
2. Cut cross sections in pith and compare the cell structure of the upper and lower surfaces. Note the *air chambers*, opening to the outer air through the pores, and containing branching *filaments* of ovoid cells with large and numerous *chloroplasts*. From your knowledge of leaf structure, what would seem to be the functions of the air chambers and green filaments? Study the structure of the thallus below the air chambers.
3. Examine the attachment and structure of the fringes and rhizoids.

Draw a cross section illustrating the above points. Microtome sections of the thallus (Sec. 212) will show certain details more clearly, as, for example, the structure of the pores, but they should be used only in comparison with sections of the living plant, in which the distribution of the chlorophyll-bearing tissue may best be studied.

C. *The cups or cupules.*[1]

1. Draw a *cup* somewhat magnified as it appears on the surface of the thallus, showing the *buds*, or *gemmæ*, within. Are cups found on the same thallus with the stalked disks and umbrella-like structures?
2. Examine a bud under m.p., noting the two notches on opposite sides, which are *growing points*, and the *scar* where it was attached to a stalk.
3. Search for young *Marchantia* plants developing from buds.
4. Section a cup in pith, and study the development of the buds and their attachment.

[1] *Lunularia*, a relative of *Marchantia* and frequently common in greenhouses, has a cup of quite different form, but otherwise agrees closely with *Marchantia* and may be substituted for it.

D. *The antheridia, or male sexual organs.* These are borne on stalked disks, with scalloped margins, and are called *antheridial receptacles,* or *antheridiophores.*

1. Draw an antheridial receptacle, showing its relation to the thallus and the number and form of the lobes of the disk. Where is it situated with reference to the midrib of the thallus?

2. Section the disk in pith. Note the *antheridia* situated in cavities among the irregularly shaped air chambers. Where are the youngest antheridia found? Make a semi-diagrammatic sketch showing these points.

3. Study the structure of an antheridium. Observe the short *stalk* and the cellular *sperm case* above. If the antheridia are not sectioned, crush and note the contents of older structures which will probably show the minute developing *sperms.* Staining the material with iodine may bring out some points more clearly.

4. Microtome sections of the antheridial receptacles (Sec. 212) will give abundant stages in the development of the antheridia and sperms, and show clearly the structure and arrangement of the *sperm mother cells* within the mature antheridium.

E. *The archegonia, or female sexual organs.* These are borne on stalked structures, with long, finger-like processes arranged like the ribs of an umbrella, and are called *archegonial receptacles,* or *archegoniophores.* Are antheridial and archegonial receptacles ever found on the same plant? Are cups found on these plants?

1. Draw an archegonial receptacle, showing its relation to the thallus and the number and arrangement of its finger-like processes. Where is it situated with reference to the midrib of the thallus?

2. Draw a view of the receptacle as seen from below, choosing an old specimen. Older material will present *sporophytes,* frequently called the *fruits* of the liverwort, in

various stages of development. Observe their arrangement in lines between *fringes*. Where are the oldest sporophytes situated?

3. Make sections in pith across rather young or medium-sized receptacles. Some of them will show the flask-shaped *archegonia* of various ages. Note the *swollen base*, or *venter*, and the *neck*. If the archegonium be not fully mature, a line of *canal cells* is to be seen in the neck, terminating in the large *egg* within the swollen base. Illustrate these points in figures.
4. Hunt for older stages following the opening of the neck and fertilization of the egg. Note the *shriveled neck* and much enlarged base, which now contains a developing sporophyte. The older fertilized archegonia become surrounded by an open, sac-like envelope (perianth) which develops at their base.
5. Make sections of the stalk and examine under m.p., noting the two *grooves* containing *rhizoids*. Follow these grooves up and down on the stalk, and trace them to the point where the stalk joins the thallus. What is the significance of the rhizoids in the grooves? What relation does the stalk bear to the thallus?
6. Microtome sections of the archegonial receptacles (Sec. 212) are almost essential to an understanding of the development and arrangement of the archegonia and their minute structure. They also show stages in the development of the sporophyte.

F. *The sporophyte.* Section old archegonial receptacles. Also pick off some of the sporophytes which have opened and are discharging spores, and mount them entire. Note and show in drawings:

1. That the mature sporophyte consists of a *spore case* borne on a *stalk* attached by a *foot* to the base of the archegonium. The latter has been ruptured by the development of the sporophyte.
2. That the spore case at maturity opens and discharges the *spores* which lie among a tangle of spirally marked filaments called *elaters*.

3. Microtome sections of the sporophytes (Sec. 212) are necessary for a study of the details of development and spore formation and the relation of the foot to the tissue at the base of the archegonium.

REFERENCE. Campbell, 23.

QUESTIONS. What are the advantages in the form and position of the thallus of *Marchantia?* What are its life habits? What are probably its most effective means of multiplication? What great advance in structure is presented by the sexual organs over those of the algæ generally? Under what conditions are the sperms set free and the eggs fertilized? What new features are introduced by the development of the egg after fertilization, — features not generally present in the algæ? Are there spores among the algæ comparable to the spores of *Marchantia?* Is it a new type of spore in plant evolution? Describe the life history and distinguish between the sexual phase, or *gametophyte*, and the asexual phase, or *sporophyte*. What is the physiological relation of the sporophyte to the gametophyte? Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to designate gametophytic and sporophytic phases respectively (App. 18). Construct a life-history formula which will express this succession (App. 18).

125. Porella, a leafy liverwort. *Frullania*, *Radula*, or other types may also be studied by a similar outline. Examine living material and describe the life habits. Note the dying away of older parts of the plants.

A. *General morphology.* Study and compare the two surfaces of the plants.

1. Draw the upper surface. Note the stem bearing two rows of overlapping leaf-like *scales*, generally called *leaves*, at the side. Is the branching regular? Observe the bud-like structure of the *growing point.*
2. Draw the lower surface. Note the third row of small scales or leaves (amphigastria) somewhat irregularly distributed along the stem; also show the under side of the lateral rows. Search for *rhizoids.*

B. *Vegetative structure.*

1. Mount some of the leaves in water. Note the simple cell structure. Are these leaf-like scales related, that is, homologous, to the leaves of ferns and seed plants? Draw a portion of the leaf and show details of cell structure, arrangement of chloroplasts, etc.
2. Examine the *rhizoids*, if found, and compare with those of *Marchantia*. Draw.
3. Lengthwise microtome sections of the growing points will show the method of growth from an apical cell and the development of the leaves.

C. *The antheridia, or male sexual organs.* These are found in *Porella* on small special branches at the side of the stem near the tip of the plant. Draw an *antheridial branch* or group of branches.

1. Tease the leaves of an antheridial branch apart, under a dissecting microscope if possible, and find the *antheridia*. How are they borne with reference to the stem and leaves? Show their position in a diagram.
2. Draw an antheridium, noting the long *stalk* and the *sperm case*. Crush older antheridia and observe the contents with developing *sperms;* stain with iodine.
3. Lengthwise microtome sections of the antheridial branches (Sec. 212) show clearly the cell structure of the antheridia and their development, and also the development of the sperms.

D. *The archegonia, or female sexual organs.* Search plants, not bearing antheridia, for short lateral branches in which the rather large leaves form close tufts. These are *archegonial branches.* The sexual organs of *Porella* are therefore on separate plants, male and female, and the genus is therefore *diœcious.*

1. Dissect these branches carefully and note a sac-like envelope (perianth) around a terminal group of *archegonia.* Diagram their position.
2. Tease the archegonia free from the surrounding leaves and envelope and draw one or more. Note their form in comparison with the archegonia of *Marchantia.*
3. It will frequently be found that one of the archegonia has been fertilized and that a young sporophyte is present in its much enlarged base, now called the *calyptra.*

E. *The sporophyte.* Old sporophytes, also called fruits, may be found at the tips of old archegonial branches. Dissect away the leaves and envelope around the base. Note:

1. The relatively long *stalk* attached to the tip of the sexual plant (gametophyte) by a *foot.*
2. The terminal *spore case* generally split lengthwise into four parts.

3. *Spores* and *elaters* frequently held within the split spore case. Draw a sporophyte and details of the spores and elaters.
4. Lengthwise microtome sections of archegonial branches (Sec. 212) give extremely interesting slides of various stages in the development of the sporophytes, showing the foot and details of the spore case when the latter is still contained within the archegonium (see *Principles*, Fig. 256). Note the four-lobed *spore mother cells*, or the groups of four spores, *tetrads*, produced by them. Compare this sporophyte with that of *Marchantia*. Draw its characteristic features in detail.

REFERENCE. Campbell, 23.

126. Anthoceros, the horned liverwort. This type is chiefly interesting for the sporophyte.

A. *The sexual plants, or gametophytes.* Note the relatively small thallus, lobed but unbranched and with an irregular margin. Contrast its simplicity with the gametophytes of *Marchantia* and *Porella*. Make a habit sketch to show these points, together with the elongated sporophytes which rise from the thallus.
   1. The vegetative structure may be studied from cross sections cut in pith. Observe the simple cell structure, each cell containing a single large *chromatophore*. Note the cavities on the lower side generally containing colonies of the blue-green alga, *Nostoc*.
   2. The sexual organs are best examined from microtome sections and form a detailed study.

B. *The sporophyte.* Observe the sporophytes of various ages developing upon the gametophyte, rising out of cylindrical collar-like outgrowths of the gametophytes.
   1. Study and draw the oldest sporophytes, noting how they split into two parts, which separate, and the delicate thread of shriveled sterile tissue (columella) which rises between. Pick such a sporophyte off and mount entire. Where are the ripe spores formed? Examine the surface for pores, or *stomata*, with *guard cells*.
   2. Lengthwise microtome sections of the sporophytes (Sec. 212) including portions of the thallus will show the remarkable *foot*, *region of growth*, and the cylinder of *spore-producing* tissue (archesporium), with stages in the development of the *spores* and other details of cell structure of great interest (see *Principles*, Fig. 258).

This sporophyte is the most interesting of any in the liverworts because of a number of features suggestive of life habits in higher plants. Chief among these are the stomata, the long period of growth and fructification, and the large foot drawing water from the gametophyte.

REFERENCE. Campbell, 23.

THE MOSSES, OR MUSCI

127. Sphagnum, the peat moss. Study the life habits, tufted growth, and ability to absorb water. Explain the latter property after a study of the cell structure.

A. *General morphology.* Note:

1. The main stem.
2. The lateral *branches.* Compare their arrangement at the top of the stem and below in a species that grows up into the air. What two forms of branches are present and what are the factors that determine their direction of growth?
3. The leaf-like *scales* or *leaves.*

Illustrate these points in a habit sketch.

B. *Vegetative structure.* Tease out the tip of a branch and obtain the youngest leaves possible. Compare their structure with that of the mature leaf. Make a series of drawings under h.p., showing this cell structure in young and old leaves. They should make clear:

1. The significance of the large empty cells, *tracheids*, found in the older leaf-like scales. What is the structure of these cells with their rib-like thickenings and large circular openings? Trace their development from the cells of young scales.
2. The structure of the green framework which surrounds the empty cells in old leaves. Of what is the framework composed? Trace its development from the cells of young scales.
3. Examine the cell structure of the stem. Are empty cells, *tracheids*, found there?

C. *The sexual organs, antheridia and archegonia.* These are so rarely found, since they are generally formed early in the spring or late in winter, that their study is not practicable in a general course.

D. *The sporophyte.* Note the groups of *sporophytes*, also called *fruits* of the moss, situated at the tips of the tufted branches. Make a habit sketch. Study:

1. The large globular spore case, opening by a lid, and situated at the end of a rather thick *column* (pseudopodium).
2. Lengthwise sections will show that the column is not a part of the sporophyte but an outgrowth from the gametophyte. The sporophytes have no stalk but are attached to the column by a large *foot* (see *Principles*, Fig. 260, *B*). The spores are developed in a dome-shaped spore-producing tissue (archesporium). Draw characteristic features of the sporophyte in detail.

Reference. Campbell, 23.

128. Funaria, Mnium, Atrichum, or other common moss.* *Polytrichum* has some advantages as a type on account of its size, but the life history of a moss is more easily studied from some of the smaller forms. Study the life habits of the type examined and of such other forms as may be available (App. 19).

A. *The leafy moss plant.* Note :

1. The stem, bearing *leaf-like scales* generally called *leaves.* What is their arrangement around the stem ?
2. *Rhizoids* attaching the base of the plant to the substratum.
3. The long-stalked *sporophyte,* also called the *fruit* of the moss, arising from the tip of the moss plant and terminating in the large swollen *spore case.* If the spore case is mature, cut it open to obtain the *spores,* or, if open, shake out the spores.

Show the above-given points in a habit sketch.

B. *The protonema and rhizoids.* Carefully wash away the earth around the base of a moss plant in a watch glass of water and mount it with any accompanying green filamentous growth. Examine under m.p.

1. Note the filaments, green and brown, attached to the base of the moss plant. The brown filaments are called *rhizoids* and were once green, but the cells have largely lost their protoplasmic contents and the cell walls have become brown. The green filaments have the same structure as other green filaments, which are to be found growing freely over the substratum. They really form a part of what is called the *protonema.*
2. The protonema first arose from the germination of spores developed in the spore cases of sporophytes, but additional protonema may be developed from the base of the moss plants. Draw the green and brown filaments under h.p., comparing their cell structure carefully.
3. Search for small *buds* which develop on the protonema and grow into the leafy moss plants. Draw stages in their development if possible.

4. Cultures of moss spores sown on earth (Sec. 206) will give a thick growth of protonema which, when examined at the proper stage, will show abundant buds and young leafy moss plants.

C. *Vegetative structure of the leafy moss plant.*

1. Mount a single leaf and examine under m.p. Note the line of elongated cells in the middle region, constituting a simple *midrib*, and the simple cell structure of the expanded portions on either side. Show these points in an outline sketch.
2. Examine a group of cells, if not previously studied (Sec. 62), for the details of cell structure, chloroplasts, nucleus, etc.

D. *The leafy moss plants bearing sexual organs.* The sexual organs may be found on separate moss stems, and such mosses have consequently been called *dioecious*. However, in some species the male and female stems have been found to be joined at the base, so that they are really branches of the same plant, which is consequently monœcious. Male and female stems may also arise from the same protonema.

1. *The antheridial, or male, stems.* These are generally smaller than the female, and the leaves at the top of the stem form an expanded *rosette* surrounding the cluster of *antheridia*. Note the color of the cluster of antheridia and their surrounding leaves. Draw an antheridial stem to show these points.
2. *The archegonial, or female, stems.* These are generally larger than the antheridial stems, and the leaves at the top are closely rolled around one another. Search for such stems in a mat of mosses bearing mature antheridial stems. Tease the leaves apart carefully at the tip under a dissecting microscope, thus exposing the group of *archegonia*. Make a semidiagrammatic sketch of the tip of the plant, showing the relation of parts.

E. *The antheridia, or male sexual organs.* Tease a cluster of antheridia apart and mount. Note:

1. The *antheridia*, elliptical, cellular structures with short stalks. The upper part is a *sperm case* and opens at the end by the swelling and rupture of a special group of cells. Draw a mature antheridium and then crush, if possible, to study its contents of developing *sperms*. Stain the crushed preparation with iodine, which may show some details of the structure of the sperms.
2. Green filaments, *paraphyses*, among the antheridia and rising above them. Observe the form and contents of the cells and draw.

Make a semidiagrammatic sketch of how a lengthwise section of the tip of an antheridial plant would appear, using prepared slides when possible, as described in 3.

3. Lengthwise microtome sections of the tips of antheridial plants (Sec. 212) present stages in the development of the antheridia and details of their cell structure and the development of the sperms.

F. *The archegonia, or female sexual organs.* Tease apart the enveloping leaves around the end of an archegonial plant. Note:

1. The stalked archegonia with very long necks. Older stages will show the necks open above and the *eggs* in the swollen bases. Younger stages will show unopened necks with the row of *canal cells*. Draw.
2. Delicate filaments, *paraphyses*, among the archegonia.

Make a semidiagrammatic sketch of how a lengthwise section of the tip of an archegonial plant would appear, using prepared slides if possible, as described in 3.

3. Lengthwise microtome sections of the tips of archegonial plants (Sec. 212) present details of the structure and development of the archegonia and frequently of the young sporophytes.

G. *The development of the sporophyte.* Study material in which developing sporophytes are still contained within the enlarged sac-like archegonium, now called the *calyptra*. Split the calyptra with a point of a needle and remove it from the needle-shaped *sporophyte*. Pick the young sporophyte

off from the leafy moss plant, or *gametophyte*, and mount entire. Note and draw:

1. The *foot* at the base of the sporophyte which was imbedded in the tissue of the gametophyte.
2. The *growing point* at the tip of the sporophyte. The sporophyte should be turned on the slide so that the growing point under h.p. shows the large, wedge-shaped *apical cell* and the series of *segments* which are cut off from it on either side.

H. *The adult sporophyte.* In a habit sketch, if not previously drawn, show the relation of the sporophyte to the gametophyte, its long *stalk*, and the *spore case* bearing the *calyptra* like a cap at the end. Select a sporophyte in which the spore case is still unopened and covered by the calyptra.

1. Remove the calyptra and under a hand lens note the *cover*, or *operculum*, over the cavity of the spore case and the position of a *ring*, generally present just below the cover.[1] Draw.
2. Pick off the cover and ring. Mount in water and draw. Note the cell structure of the ring and its behavior when wet.
3. With a razor cut off the end of the sporophyte after the cover has been removed and mount in water. A circle of *teeth* is generally evident in the preparation, all pointing inward in a regular arrangement. Underneath the teeth may frequently be found another circle of delicate *segments* similar in form and arrangement to the teeth. Count the teeth and segments. Show these points in a figure. Note the *spores* in the spore case. The circles of teeth and segments constitute the *peristome*. If the teeth are not clearly shown, examine material as described in 4.

[1] Species of *Bryum* are especially favorable for the study of the ring and peristome (see *Principles*, Fig. 269).

4. Select an old sporophyte in which the spore case has ripened and is open. Carefully split it lengthwise with a needle, after soaking in hot water or a dilute potash solution, or mount entire. Note the circle of teeth and cilia around the opening, and the spores generally present. Draw these structures.

I. *The structure of the spore case.* Make lengthwise sections in pith of the unopened spore case.

1. Slices from the surface rather low down on the spore case are likely to give surface views showing pores, or *stomata*, with two *guard cells*. Draw. Compare these structures with stomata which may have been studied on the leaves of seed plants.
2. Median sections present a cylinder of *spore-producing tissue* (archesporium) inclosing a large pith-like region, or *columella*. A large air space crossed by filaments lies between the tissue in the interior of the spore case and the outer wall (see *Principles*, Fig. 270, *E*).

   Show the structure of the median section in a semi-diagrammatic figure.

3. The histology of the spore case can best be studied in lengthwise microtome sections.

Reference. Campbell, 23.

Questions. What are the life habits of the mosses? Why do they so frequently grow together in large tufts or mats, and what are the advantages of these growth habits? What are the advantages of their erect stems and the arrangement of the leaf-like scales? What are the means of multiplication? What great advances in structure are shown by the sexual organs over those of the algæ? Under what life conditions are the sperms set free and the eggs fertilized? Trace the development of the egg after fertilization into the stalked structure bearing the spore case. Why is this structure considered an asexual generation (sporophyte) distinct from a

sexual generation (gametophyte)? Are there spores among the algæ comparable to the moss spores? Is it a new type of spore in plant evolution? What is the physiological relation of the sporophyte to the gametophyte? What important advance in the structure of the moss sporophyte over that of *Marchantia* is indicated by the presence of stomata? In what other respects does the moss sporophyte show advances over that of *Marchantia*? Describe the life history, distinguishing between the sexual phase, gametophyte, and the asexual phase, sporophyte. Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to designate the gametophytic and sporophytic generations respectively (App. 18). Construct a life-history formula that will express this succession (App. 18).

THE FERNS, OR FILICINEÆ

129. Field work on the ferns, horsetails, club mosses, and quillworts. Representatives of these groups have well-defined habits, some living under very special life conditions and others forming striking and characteristic *associations* (see *Principles*, p. 475), such as the growths of bracken fern (*Pteris aquilina*), many horsetails, and some club mosses and quillworts. These may be studied in the field and the habits of growth and life conditions noted. The adaptations of the pteridophytes are extremely various. There are hydrophytes, as some species of *Marsilia* and *Isoetes*, and floating aquatics, such as *Azolla* and *Salvinia*. Numerous mesophytes occur especially among the ferns, as illustrated by species of *Aspidium*, *Asplenium*, and *Cystopteris*. Xerophytes are represented in the ferns by such forms as *Gymnogramme* in the far Southwest and *Polypodium incanum* in the South. *Equisetum* and certain species of *Selaginella* are excellent types of xerophytes, and some species of the latter may remain perfectly dry for long periods and will revive again when wet. Field work on the pteridophytes should be planned with reference to the ecological relations of the forms and may be accompanied with great advantage by morphological and histological studies that will show the character of the adaptations in the plant's structure. In taking up the pteridophytes the student passes to a group whose life relations approach those of the seed plants, and they may be studied in the field largely after the same methods as the latter group (see Sec. 157). The displays

offered in park greenhouses and conservatories present excellent opportunities to city classes for interesting studies on life habits, especially of tropical forms.

130. Aspidium, Pteris, Adiantum, or other common fern.** There are a number of wild and greenhouse ferns, all almost equally good for a type study (App. 20). Examine the growing fern: (1) determine the position of the stem, its habits of growth, whether upright or creeping, partly above ground, or wholly subterranean; (2) examine the arrangement and distribution of the fronds or leaves, their manner of unrolling; (3) study fruiting fronds bearing on their surface minute brown sporangia, variously arranged and protected in different species of ferns.

A. *General morphology.* Note:

1. The *stem*, if creeping called a *rootstock* (rhizome). Observe whether it is subterranean or above ground.
2. The *roots*, their distribution on the stem.
3. The *fronds*, or *leaves*, consisting of a *leafstalk* (stipe) and *blade* (lamina). Note the unrolling of the fronds. Search for the scars of old fronds formerly on the stem. The fronds of most ferns are compound, that is, divided into segments or *leaflets*. Sometimes the primary segments are divided again and these still again. Such fronds are twice or thrice *compound* or branched as the case may be. Describe the conditions in the frond of your type. Examine the *veins* in the leaves and describe their branching. Has the system of *venation* any relation to the form of the margin or the compound character of the frond?
4. The *sporangia* on the surface of the leaves. Observe their arrangement in spots or regions. Each spot is called a *sorus* and is generally partly or wholly covered by a protective membrane, the *indusium*. Do the positions of the sori bear any relation to the veins? The structure and position of the indusium is an important character in the classification of ferns.

Illustrate in a habit sketch the characteristic features of frond and stem, and draw in detail a portion of a fruiting frond, showing the venation and distribution of sporangia.

B. *The sporangia.* Scrape off some of the sporangia from a sorus and mount in water. Draw under h.p. a side view of a sporangium from which the spores have been discharged. Note and show in the figure:

1. The *stalk.*
2. The flattened *spore case* consisting of thin-walled tissue except for a row of thick-walled cells along the margin, forming the *ring* (annulus). Note the extent of the ring and the position of the thickened portions of its walls.
3. The wide rent in old and empty sporangia opposite the ring.
4. *Spores* free or inclosed in the sporangia. Draw in detail.
5. Soak some ripe, unopened sporangia in water and place on a slide without a cover glass. Watch them under l.p. as they become dry. Explain from the movements of the ring how the sporangium opens and discharges its spores.

The fact that the fern plant has no sexual organs but produces instead asexual spores, and that it alternates with a sexual generation (as will appear later), makes it a *sporophyte.* The details of spore formation show that the spores are comparable to those of the bryophytes.

6. The details of the development of the sporangia and of spore formation can best be studied from microtome sections through the sori. Species of *Aspidium* are especially favorable for this study (Sec. 212). Follow stages as outlined in *Goebel*, 16, Fig. 165. Note that the spores, sixty-four in number, are developed in groups of fours, *tetrads*, in *spore mother cells.*

C. *The cell structure, or histology, of the stem.* The fern is not a favorable subject for a detailed study of the tissues characteristic of higher plants, but certain peculiarities are important.

1. In cross sections of the stems or leafstalks note the *fibro-vascular bundles* and regions of thick-walled *rigid tissue* (sclerenchyma) differentiated from the thin-walled fundamental or *ground tissue* (parenchyma). Show their distribution in an outline drawing. The outer layer of cells will be clearly differentiated as an *epidermis* if the structure studied is aërial,—that is, above ground. Why? What is the chief purpose of the rigid tissue? What are some of the functions of the fibro-vascular bundles?

2. In cross sections of fibro-vascular bundles, under h.p., observe (1) large, thick-walled, empty cells, called *tracheids;* these with small, thick-walled cells in the interior constitute the *wood*, or *xylem;* (2) the surrounding soft, thin-walled tissue, called *bast*, or *phloëm*, containing *sieve tubes* (see *Principles*, Fig. 274); (3) the *bundle sheath* and *phloëm sheath*, two adjacent cell membranes inclosing the bast and wood.

Study these elements of the bundle in stained preparations (Sec. 212) of cross and lengthwise sections, making detailed drawings.

D. *The cell structure, or histology, of the frond.*

1. Strip off the epidermis from the lower surface of the frond and mount in water, with the outer surface of the epidermis uppermost on the slide. Note the pores, or *stomata*, with their *guard cells*, and draw these with adjacent *epidermal cells.*

2. Strip off epidermis from the upper surface. Has it stomata?

3. Sections of the blade will show the green parenchyma, *mesophyll*, and *air spaces* within the leaf opposite the stomata. The cells of the green tissue on the upper side of the frond are usually arranged in a firm layer, or *palisade.* Note that the veins are fibro-vascular bundles giving strength to the expanded blade, besides carrying water to all parts of it.

E. *The structure of the root tip.* Study lengthwise microtome sections of the *root tip* (Sec. 212). Note the *root cap* over the *growing point*, which consists of a pyramidal *apical cell* whose apex points inwards. Segments

are cut off from the sides of the apical cell to form the root and from its base to form the root cap. Trace the history of these segments in your preparation. Draw.

F. *The germination of the spores.* Examine a two- or three-weeks-old culture of fern spores sown on earth or on old, dirty flowerpots (Sec. 208). Spores are to be found in various stages of germination. The structure developed from the spore is called a *prothallium.* It will, however, become apparent from the study of a mature prothallium that the structure is a *gametophyte.* Note:

1. The ruptured spore and protruding green filament.
2. The change of the tip of the filament into a flat plate of cells by the formation of oblique walls, which cut out a wedged-shaped *apical cell.*
3. The development of *rhizoids.* Where?
4. As development proceeds the apical cell becomes situated in a *notch* on account of the growth of the cells on both sides, and the prothallium takes a heart-shaped form.

Draw a series of stages in the germination of the spores and development of the prothallia.

G. *The mature prothallia, or gametophytes.* Examine a large, heart-shaped prothallium six or more weeks old. Observe its thalloid structure, the angle at which it rises from the substratum, the position of the notch. Mount with the lower side uppermost. Note:

1. The position of the rhizoids.
2. Small, round *antheridia,* many of them probably open and brownish, situated at the lower or posterior end of the prothallium.
3. The projecting necks of *archegonia* just back of the notch on a thicker region of the prothallium called the *cushion.*

Show the position of these structures in an outline sketch.

The prothallia are *gametophytes,* since they produce sexual organs, arise from the asexual spores of the *sporophytes* (fern plants), and alternate with this asexual generation.

H. *The antheridia, or male sexual organs.* These are frequently more easily studied on smaller *dwarf* prothallia, especially those which have been growing crowded together, and are consequently irregularly developed and devoted entirely to the production of antheridia (not a strictly normal condition). Examine under h.p. mature antheridia situated near the edge of the prothallium, so that they may be seen in side view. Note and illustrate in figures:

1. The funnel-shaped *basal cell.*
2. The *ring cell* surrounding the side of the antheridium.
3. The disk-shaped *cover cell.*
4. The central group of developing *sperms,* or *sperm mother cells.*
5. Mature antheridia, especially those borne on somewhat dry prothallia, when mounted in water, will open and discharge the *sperms.* Watch their escape and movements and then stain with iodine.
6. Observe the coiled, spiral *body* of the sperm, the numerous *cilia* at the pointed end, and generally a *vesicle,* the remains of the sperm mother cell, at the larger end.

I. *The archegonia, or female sexual organs.* Mount a large, heart-shaped prothallium with the lower side uppermost, together with several dwarf prothallia, some of whose antheridia are likely to discharge sperms. Study the necks of the archegonia projecting from the cushion back of the notch. Note how they curve backward and downward from the cushion. Some of the necks are likely to open. Observe that sperms swimming freely in the water gather around the open necks and enter them. Illustrate these features.

1. The structure of the archegonium can best be studied from microtome sections cut lengthwise of the prothallium (Sec. 212). The base of the archegonium with the *egg* lies imbedded in the prothallium. There are only two or three *canal cells.*

J. *The young sporophytes.* In cultures of prothallia two or three months old, which have been watered from

above, observe the development of young fern plants, or *sporophytes*. Note the position of the small first leaf rising generally through the notch of the prothallium. Remove such a young sporophyte with the attached prothallium, wash the dirt from the root, and draw under low magnification.

1. The development of the sporophyte can best be studied from microtome sections of material cut as described in I, 1. Younger stages show the early divisions of the egg into four regions which develop respectively into a *stem*, *root*, *first leaf*, and large *foot* attaching the young sporophyte to the gametophyte. Later stages will show the gradual differentiation of these four regions into the organs named above. The large foot brings the sporophyte into intimate physiological relations with the gametophyte (see *Principles*, Fig. 279, *A*), from which it may obtain food until able to live independently by the development of a root-and-leaf system.

References. Campbell, 23; and for a detailed study of the structure and life history of *Pteris aquilina*, see Sedgwick and Wilson, *An Introduction to General Biology*, 1895.

Questions. What are the growth habits of the ferns? Are they long-lived? What are the means of reproduction? Are there other means besides spores? Why are the spores of ferns and bryophytes comparable to one another? Trace the history of the germination of fern spores. Are the prothallia generally long-lived? Why are they gametophytes? Are the gametophyte and sexual organs simpler in structure than those of the mosses and most liverworts, and in what respects? Explain how this can be in a group (pteridophytes) higher than the bryophytes. Under what conditions are the eggs fertilized? What are the life habits at this time? What are the chief advances of the sporophytes of ferns over those of bryophytes? Describe the life history and distinguish between the sexual phase, gametophyte, and the asexual phase, sporophyte. Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to

designate the gametophytic and sporophytic generations respectively (App. 18). Construct a life-history formula that will express this succession (App. 18).

131. Fronds, vegetative leaves, and spore leaves, or sporophylls. Study the fronds of such ferns as *Onoclea sensibilis*, or *O. struthiopteris*, or *Osmunda cinnamomea*. *Osmunda regalis* and *O. Claytoniana* illustrate the points outlined below to a partial extent. Herbarium material, — that is the fronds mounted on sheets, — may be used. Observe that there are two forms of fronds:

A. *Vegetative fronds*, which may be called *vegetative leaves*, or simply *leaves*.

B. *Spore-bearing fronds*, which are called *spore leaves* or *sporophylls*. The sporophylls are devoted almost entirely to the work of spore production. They are not expanded; there is relatively little green tissue, and they are not well suited to the work of photosynthesis. Thus the fronds of these ferns have become differentiated into two sets, one devoted to the purposes of spore production and the other to vegetative activities.

Examine a series of leaves illustrating the general conditions stated above. Compare them and draw. Search for leaves with mixed characters, that is, in parts devoted to spore production, and in other parts strictly vegetative. These are not uncommon, and their study makes clearer the relationship between the vegetative leaves and the spore leaves or sporophylls. *Ophioglossum*, *Botrychium*, *Aneimia*, and *Lygodium* illustrate excellently the mixed characters noted above, but material is less likely to be available. Some of the common ferns — as the Christmas fern (*Polystichum acrostichoides*) — also show the same tendencies.

132. Marsilia, a water fern.* Study the life habits of the plant as it grows in the water or in wet situations.

A. *General morphology.* Note:

1. The creeping stem, or *rootstock* (rhizome).
2. The four-parted *leaves*. Contrast their veining with that of the clover leaf.
3. The fibrous *roots*.

* To the Instructor: If only one heterosporous pteridophyte can be studied in the laboratory, it is perhaps better that the form be *Selaginella*. But the ease with which the spores of *Marsilia* may be germinated and the gametophytes obtained, together with the excellent study of sperms and the young sporophyte, which is offered, make it an especially attractive type for study, and it should be included in a general course whenever possible.

4. The *spore fruits* (sporocarps) borne in groups on short stalks, attached to the base of the leaf. The spore fruits are modified portions of leaves, which are consequently highly specialized sporophylls.

Illustrate these features of the general morphology in a habit sketch.

B. *The spore fruit, or sporocarp.* Spore fruits of *Marsilia vestita* open more readily in water and are generally more favorable for this study than those of *M. quadrifolia*. Chip the edge of a spore fruit with the point of a heavy knife and place in water. It will probably open in an hour or more, splitting along one side like a pod. Note:

1. The emergence of a worm-like gelatinous structure bearing elliptical spore masses, which are really groups of sporangia and consequently *sori*. Make a drawing to show their appearance after emerging from the spore case. Split a dry spore fruit lengthwise and observe the manner in which the sori are arranged within. Illustrate.
2. Examine a sorus under l.p. Note the two sizes of spores, the larger called *megaspores* and the smaller *microspores*. Show their arrangement in the sorus.
3. If the spore fruit has not been in the water too long, the microspores will be found in groups of sixty-four, held within a very delicate tissue which represents a *microsporangium*. In this connection it is important to remember that the common ferns produce sixty-four spores in their sporangia.

C. *The microspores and megaspores.*

1. Draw the two forms of spores side by side to show comparative size. Note the slight protuberance at one end of the megaspore.
2. Crush the megaspore and stain the contents with iodine. What are the large grains?

The spores begin to germinate at once, and after eighteen or twenty hours develop small gametophytes of two forms: (1) *male*

gametophytes, developed by the microspores; and (2) *female gametophytes*, developed by the megaspores. The differentiation of spores into two sizes, microspores and megaspores, which develop respectively male and female gametophytes, is called *heterospory*.

D. *The male gametophyte.* This small structure can best be studied by means of microtome sections (see *Principles*, Fig. 282, *A*). Living material will, however, show the ruptured microspore, a protruding cellular papilla, and, at the proper stage, developing *sperms* within. Draw such germinating spores.

1. *Sperms* are generally to be found swimming about in the water, especially in the vicinity of megaspores, which by this time have developed *archegonia*, formed singly at one end of the spore. Excretions from the archegonium exert an attractive influence, called *chemotaxis*, upon the sperms.
2. Stain the sperms with iodine. Note the spiral *body*, like a long corkscrew, and the numerous *cilia* distributed over it. A *vesicle*, frequently to be found at the larger end of the spiral, is the remains of the *sperm mother cell*. Draw the stained sperms.

E. *The female gametophyte.* This consists of a short-necked *archegonium* developed at the end of the megaspore, which was marked by the slight protuberance. Microtome sections are necessary to show its detailed structure (see *Principles*, Fig. 282, *C*). In the living material note and show in a figure:

1. The opening into the short *neck*.
2. The arrangement of *slime* around the archegonium end of the megaspore in which sperms are frequently caught.
3. The swollen base of the archegonium containing the *egg*.

Note the relatively small amount of chlorophyll in the cells of the female gametophyte. Where does it get the food necessary for its development?

F. *The young sporophyte.* The sporophyte begins to develop at once from the fertilized egg. The early stages of its

division differentiate four regions, which develop respectively into *stem*, *root*, *first leaf*, and a large *foot*. These stages can only be studied from microtome sections. The first leaf and root grow rapidly and in a few days break through the much-enlarged archegonium (calyptra).

1. Watch the development of the sporophytes in a culture of spores. When about one week old draw a megaspore with the attached sporophyte under l.p. Determine by the root cap which portion is root and which leaf. The stem lies at the base of the leaf. Where is the foot?
2. Crush the megaspore and observe the condition of its food contents. What has furnished the food for the development of the sporophyte?

REFERENCE. Campbell, 23.

QUESTIONS. What are the growth habits of *Marsilia?* What are the life conditions governing the germination of the spores and development of the gametophytes? What is heterospory? What are the advantages in the differentiation of a large megaspore richly supplied with food? What are its advantages in giving the sporophyte a better start in life? Describe the life history, distinguishing between the sexual phases, male and female gametophytes, and the asexual phase, sporophyte. Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to designate the gametophyte and sporophyte generations respectively (App. 18). Construct a life-history formula that will express this succession (App. 18).

THE HORSETAILS, OR EQUISETINEÆ

133. Equisetum arvense, the field horsetail. This is the commonest of the horsetails and is abundant along railroad tracks, roadsides, river banks, and bare northerly slopes. The *spore-bearing shoots* appear early in April and are followed shortly by

the *vegetative shoots*. Study the plant in the field and dig up the underground *rootstocks* (rhizomes). Note their extent and the manner in which the plant establishes itself in the soil, its luxuriance in waste ground, and its endurance of drought. It is in structure an excellent illustration of a *xerophyte* (see *Principles*, p. 459), showing very striking adaptations to its life habits.

A. *General morphology.* Study entire plants, including the rootstocks. Well-mounted herbarium sheets of plants collected when the spores are shed, and another set collected about a month later are excellent for comparative study. In plants collected in April with spore-bearing or fertile shoots note:

1. The upright, pale yellowish, unbranched shoots bearing cones at the tip composed of six-sided scales. These shoots are the *spore-bearing*, or *fertile shoots*. Examine their *jointed* structure, and in preserved material pull the joints apart.
2. Note the toothed sheaths at the joints (nodes). Each tooth represents a *leaf*.
3. Observe the developing, branched, vegetative shoots. Have they the same jointed structure with toothed sheaths as the spore-bearing shoots?
4. Trace the spore-bearing shoots and also the developing vegetative shoots to the creeping *rootstock* (rhizome). What is the structure of the rootstock? Has it joints? sheaths? Where do the roots arise? Note the *tuberous bodies*, if present.

Illustrate the above features in a habit sketch, with such details of the joints and sheaths as are necessary to make clear the fundamental morphology.

In plants collected somewhat later, with well-developed vegetative shoots, note:

5. That the spore-bearing shoots have died.
6. That the vegetative shoots have developed into tall green, much-branched stalks. How do they feel to the touch?

7. Study the jointed structure of the branches, the sheaths, the method of branching. What part of the plant performs the work of photosynthesis?

Draw a portion of the vegetative shoot to illustrate the above points.

B. *The cone and its scales, or sporophylls.* Study from preserved material.

1. Draw a cone in detail (if not drawn under A), showing the arrangement of the hexagonal *scales* in circles around the stem. Why should their form be six-sided? Compare the circles of scales with the nodes or joints of the stem bearing circles of leaves forming sheaths.
2. Cut off several scales, noting the central stalk bearing the six-sided plate and the sac-like *sporangia* hanging down from the plate. Draw two side views of the scales as seen somewhat obliquely from above and below. The scales are highly specialized *spore leaves,* or *sporophylls.* What are some of the reasons why they should be so considered?

C. *The sporangium and its spores.* Split a sporangium open with the point of a needle. Examine under h.p. Note:

1. The *spores,* each bearing four filamentous *elaters,* developed from four segments of the outer spore wall, which separate from one another and the spore except at one point.
2. Allow the spores to become dry and note the position of the elaters. Draw.
3. Breathe gently on the dry spores or moisten them and observe the behavior of the elaters. Draw various spores.
4. Study the structure of the sporangium wall, the cells of which are irregularly thickened. Draw a portion under h.p. How does the sporangium wall rupture? What mechanical forces are at work to make it split open?

D. *The cell structure or histology of the stem.* Cut sections across the stem between the nodes. Observe under l.p. the

air spaces, the distribution of *green tissue*, the *rigid tissue* around the outside, the small *fibro-vascular bundles*. Show these structures in an outline sketch.

1. The detailed structure of the stem is complicated and highly specialized for its work of photosynthesis under severe xerophytic conditions. There are many interesting adaptations to these ecological relations, as shown by the position of the stomata protected within the lengthwise grooves, the heavy layers of thick-walled tissue outside of the green tissue, etc. The study of these adaptations is very interesting, but rather special, requiring well-cut sections.

E. *The growing points of stems and roots.* These are occupied by remarkable, large *apical cells* whose structure and activities are best studied by means of microtome sections. They are among the best illustrations of growth from apical cells.

F. *The gametophytes.* These can be obtained only by sowing spores when very fresh. The prothallia are irregularly lobed and the antheridia and archegonia are generally developed on separate plants (see *Principles*, Fig. 285).

References. Campbell, 23; Goebel, 16.

Questions. What are the structural characters of the horsetails especially adaptive to severe conditions of heat and drought (xerophytic conditions)? Where is the work of photosynthesis performed? Have the leaves anything to do with it? Have the leaves any very obvious functions? Why are they present especially on the underground rootstock? What is the form and structure (morphology) of the cone? What are some of the advantages of the elaters on the spores? What are the advantages in the spores clinging together so that they germinate in groups?

THE CLUB MOSSES, OR LYCOPODINEÆ

134. Lycopodium, the lycopod, or club moss. Species of *Lycopodium* with well-differentiated cones, such as *L. annotinum*, *L. complanatum*, *L. clavatum*, etc., furnish the best material for type studies. Observe when possible the life habits, noting the creeping stems from which arise the upright branches bearing cones.

A. *General morphology.* Well-mounted herbarium sheets are excellent for this study. Note:

1. The *upright stems*, with spirally arranged, needle-shaped *leaves.*
2. The long terminal *cones*, composed of spirally arranged *scales* (*sporophylls*).
3. The *creeping stems* with leaves similar to those of the upright stems.
4. The rather infrequent *roots.*

Illustrate the above features in a habit sketch.

B. *The cone and its scales, or sporophylls.* Examine preserved material.

1. Draw a cone in detail, showing the spiral arrangement of its scales (sporophylls) if not illustrated under A.
2. Cut off several scales and draw one as seen from the inside, showing the large sporangium at its base.
3. Construct a diagram illustrating the attachment of the scales to the axis of the cone and the way in which they overlap one another.

The scales are specialized spore leaves, or sporophylls. What are some of the reasons why they should be so considered?

C. *The sporangium and its spores.* Split a sporangium open and note the immense number of minute *spores.* Draw a group. What is the significance of the angles along one side? Like the spores of the bryophytes and pteridophytes generally, they are developed in groups of four, *tetrads*, from *spore mother cells.*

D. *The cell structure, or histology of the stem and leaf.* This is a profitable but detailed study.

1. Cut cross sections of the stem. Note the *epidermis*, the thick cortical regions of *ground tissue*, the vascular strands called *leaf traces*, leading out to the leaves from the central *fibro-vascular bundle.* In the fibro-vascular bundle observe the more or less parallel regions of *wood* (xylem), composed of large *tracheids*, and *bast* (phloëm) within the *bundle sheath.* Draw the details of cell structure in a series of figures from cross and lengthwise sections.
2. Examine the surface of the leaves for *stomata.* Cross sections of the leaves will show their simple cell structure.

E. *The germination of spores, and the gametophytes.* Gametophytes have not been found in America and the spores have not been germinated beyond the first few cell divisions. The propagation of plants is chiefly by the branching of stems which separate as older parts die away, and in some species by peculiar vegetative *buds.*

Reference. Campbell, 23.

135. Selaginella. Various species of *Selaginella* have quite different habits of growth and arrangements of leaves and branches.

S. rupestris is generally the most available of our native species, but *S. opus* is often easily obtained. *S. Kraussiana* is a delicate form frequently cultivated in conservatories. *S. Martensii* and other tropical species are large, erect, much-branched, ornamental plants cultivated in greenhouses, and when in good fruit are excellent for type study. A single plant in fruit will supply a large class with material, but the smaller species are also good.

A. *General morphology.* Note:

1. The character of the *stems*, upright or creeping. Describe their method of branching, generally in one plane.
2. The scale-like *leaves.* These are spirally arranged in some species (as *S. rupestris*), but in other forms are distributed in four rows and are of two sizes. What is their arrangement in the type studied? Have the stems an upper and lower side (dorsiventral symmetry)? What advantage is there in this symmetry in relation to the spreading habits of growth? Search for a very small triangular structure, called the *ligule*, at the base of the leaf. Its significance is not known.
3. The spike-like *cones* composed of crowded *scales* (*sporophylls*). How are these arranged? How many rows? What is the geometrical form of the cone?
4. The fibrous forking *roots.* In tropical species roots may be developed from the tips of special descending branches (rhizophores).

Illustrate the above points in habit sketches, paying special attention to the arrangement of the leaves on the stem.

B. *The cone and its scales, or sporophylls.*

1. Draw a cone in detail, showing the arrangement of the *scales*, or *sporophylls* (if not illustrated under A).
2. Note the *sporangium* attached at the base of each sporophyll. Are the upper and lower sporangia of the same cone alike? Cut off the scales and compare them, distinguishing between oval sporangia containing minute spores, *microspores*, and larger-lobed sporangia containing

a few very large spores, *megaspores*. These two forms of sporangia are termed respectively *microsporangia* and *megasporangia*, and the scales which bear them are *microsporophylls* and *megasporophylls*. Draw the microsporophylls and megasporophylls, viewed from the inside, showing the form of the sporangia.

3. Construct a diagram illustrating the attachment of the two forms of scales to the axis of the cone and their distribution above and below.

The scales, as stated above, are *sporophylls* differentiated into two forms. What are the reasons why they should be so considered? The differentiation of spores into two sizes, microspores and megaspores, which develop respectively male and female gametophytes, is called *heterospory*.

C. *The microsporangium and microspores.* Split a *microsporangium* open and note the immense number of minute *microspores*. Draw, under h.p., a portion of the wall of the sporangium, showing the cell structure, and a group of spores.

D. *The megasporangium and megaspores.* Split a *megasporangium* open and count the large *megaspores*. Is the number the same in all sporangia? Draw a megaspore under h.p., showing the form and markings on the wall. What is the significance of the angles on one side? Like the spores of the bryophytes and pteridophytes generally, and also like the microspores, they are developed in groups of four, *tetrads*, from *spore mother cells*. Under the same magnification draw a microspore by the side of the megaspore to show comparative size.

E. *The cell structure, or histology of stems and leaves.*

1. Cut cross sections of the stem. Note the *epidermis* and the cortical *ground tissue* surrounding two or more *air spaces* crossed by delicate filaments. In the center of each space lies a *fibro-vascular bundle* consisting of a strand of *wood* (xylem) surrounded by *bast* (phloëm). The further examination of these elements in cross and lengthwise sections may constitute a detailed study.

2. Compare the cell structure of very young leaves with that of older ones. The cells, in the younger leaves, contain a single large chromatophore, which becomes divided into a chain of segments in the older cells.

F. *The germination of spores, and the gametophytes.* The microspore produces a very small *male gametophyte*, which remains contained within the ruptured spore wall and develops two-ciliate *sperms* (see *Principles*, Fig. 290, *A*, *B*). The megaspore gives rise to a small *female gametophyte*, which emerges somewhat from the ruptured spore and develops several *archegonia* (see *Principles*, Fig. 290, *C*). The development of these gametophytes requires several weeks, and their study demands microtome sections, which are difficult to prepare, so that they can hardly be treated in an elementary course. *Marsilia* (Sec. 132) is a better type for the study of reduced male and female gametophytes associated with microspores and megaspores.

G. *The development of the sporophyte.* This study, like that of gametophytes, requires microtome sections of material difficult to obtain and to cut, and is hardly practicable for a general course. *Marsilia* (Sec. 132) is a better type to illustrate the relation of the embryo sporophyte to the female gametophyte in heterosporous pteridophytes. The embryo develops in the interior of the gametophyte at the end of a structure called the *suspensor*. Three regions are differentiated, — the *stem* with young leaves forming a bud, the *root*, and the large *foot* (see *Principles*, Fig. 290, *C*). The foot absorbs food from the tissue of the gametophyte within the megaspore. In certain species of *Selaginella*, as *S. rupestris*, the sporophytes are developed while the megaspores are still held mechanically by the sporophylls on the cone (see *Principles*, Fig. 290, *D*). This retention of the megaspores on the sporophyte is suggestive of the seed habit (see *Principles*, pp. 335 and 336).

Reference. Campbell, 23.

Questions. What are the growth habits of the type of *Selaginella* which has been studied? Are there any xerophytic adaptations? Describe any peculiarities in the arrangement of the leaves and suggest reasons therefor. What is the form and structure (morphology) of the cone? Why are its leaves called sporophylls? What is the relation between the large size of the megaspores and their production relatively few in a megasporangium? What is heterospory? What are its advantages in giving the sporophyte a better start in

life? Describe the life history, distinguishing between the sexual phases, gametophytes, and the asexual phase, sporophyte. Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to designate the gametophytic and sporophytic generations respectively (App. 18). Construct a life-history formula that will express this succession (App. 18).

136. Isoetes, quillwort. Study when possible the life habits, noting whether the form is aquatic or terrestrial.

A. *General morphology.* Note:

1. The rush-like *leaves* arranged around a very short, conical *stem*, and the cluster of forking *roots* below. Illustrate in a habit sketch. These leaves, late in the season, produce sporangia at their bases, thus becoming *sporophylls*. At such times the stem may be compared to a cone of sporophylls.
2. Strip the sporophylls from the stem. Those on the outside, and consequently lower on the stem, are likely to be *megasporophylls*, as shown by the basal sporangium containing *megaspores*. Those in the interior, and consequently higher up on the stem, are likely to be *microsporophylls*, as shown by the basal sporangium containing *microspores*.

B. *The sporophylls.* Examine the base of a megasporophyll, viewing it from the inner side. Show in a figure:

1. The large *megasporangium* containing *megaspores*, held in a hollow at the base of the leaf and partially covered by a membrane, the *velum*.
2. The *ligule*, a triangular scale situated on the sporophyll above the sporangium. Diagram the position of the ligule and sporangium as they appear in a lengthwise section of the base of the sporophyll.
3. Draw the megaspore under h.p., showing the markings on the thick wall and the angles on one side. What do the angles signify?
4. Compare the base of a *microsporophyll* with that of a megasporophyll.
5. Draw a group of *microspores* under h.p. to show their size in comparison with that of the megaspore.

C. *The structure of the leaf.* Section the leaf across and lengthwise. Note the large *air spaces* separated by partitions. Show their arrangement in an outline drawing and the small *fibro-vascular bundle* traversing the interior of the leaf. Are *stomata* present?

D. *The gametophytes.* The germination of the spores of *Isoetes* and the development of the gametophytes is even more difficult to follow than that of *Selaginella* and requires detailed study.

Reference. Campbell, 23.

THE GYMNOSPERMS, OR GYMNOSPERMÆ

137. Cycads. *Cycas revoluta* is a large form frequently cultivated in park conservatories, and may be used to illustrate the general morphology of the cycads, that is, the trunk-like, unbranched stem bearing the crown of compound leaves at the top. This cycad occasionally develops sporophylls in the greenhouses, which may then be collected and preserved for study. The carpels are developed more commonly than the stamens.

Zamia is a small cycad which grows in Florida and is an admirable type for a study of the cycad cone, together with the development of the ovules and pollen, the structure of the male and female gametophytes, processes of fertilization, and development of the embryo. Cones of *Zamia* may be readily shipped, and since the ovules retain their vitality for a considerable time they can be studied alive in the North or killed and preserved for microtome sections. *Zamia* may also be readily grown from seed in greenhouses, and will produce cones under cultivation. Its study is recommended wherever possible.

138. The pine (App. 21). Several species are available for this study, such as the Scotch pine (*Pinus sylvestris*), the Austrian pine (*P. laricio*), *P. Strobus*, *P. palustris*, or some of the scrub pines, such as *P. Banksiana*. Living material for general morphological or histological work may be obtained at any time of year. The young cones should be collected and preserved in alcohol at the time of pollination in May, when the year-old cones and two-year-old cones may also be gathered and preserved for comparison with the first. Dried, open cones and seeds should be collected late in the summer. The pine should also be studied in the field to determine its growth habits and the ecological adaptations of its foliage to conditions of severe cold and drought.

A. *General morphology.* Observe:

1. The main *stem* and *branches*, each ending in a *terminal bud;* the arrangement of the branches.

2. The very numerous *dwarf branches*, each bearing a cluster (fascicle) of two, three, or more needle-like *leaves*, according to the species studied.
3. The thin *scales* on the dwarf branches wrapped around the base of the cluster of needle leaves. Are these scales morphologically leaves? Why?
4. The bases of old *scales* spirally arranged, and covering the main branches from the axils of which the dwarf branches arise. Younger full-sized scales at the tips of the shoots. What are these scales morphologically?
5. The bud scales, or leaves, enveloping the terminal bud.

Illustrate these features in habit sketches. How many forms of leaves are there on the pine?

6. On a branch two feet or more in length note the regions that indicate the beginnings of one year's growth and the end of another's. Draw such a region and explain the peculiar arrangement of the scales upon it.
7. Observe the position of cones on the branches. How many sizes do you find and what are their ages as shown by their positions?
8. Note the branch scars on older portions of the stem and main branches.

B. *The cell structure, or histology, of the stem.* Cut cross sections of a three- or four-year-old branch. These may be stained with advantage (Sec. 212) and mounted in balsam.

1. Observe under l.p.: (*a*) the restricted region of *pith;* (*b*) the layers or rings of *wood*, or *xylem*, around the pith (what is the significance of their number?); (*c*) a layer of *bast* or *phloëm* outside of the wood and separated from it by a thin *cambium;* (*d*) the *outer bark* composed of larger cells, in places green, but on the exterior dead and forming scales; (*e*) *medullary rays* appearing as radiating lines running through the wood and bast; (*f*) *resin ducts* in the wood and outer bark. Are the medullary rays of the same length? Do any of them penetrate to the pith?

   Show the position of these tissues in an outline sketch.

2. Study the structure of the wood in the region between the growth of two successive years. In a detailed figure show the form of the

empty wood cells and explain differences in size. Also include in the figure a medullary ray, the cells of which have dense protoplasmic contents, and also a resin duct. Note the cross sections of *pits* in the wood cells, which will be better understood after the study outlined in C. In what faces of the cells are they found?

3. Study the region of the cambium and show the form of its cells in a detailed figure, together with some of the wood on the inside and the bast on the outside, including a medullary ray. The bast is composed for the most part of *sieve tubes*. What are the peculiarities of the cambium tissue characteristic of a region of growth?
4. Study the outer bark, showing in figures (*a*) the form of the old bast cells having a crushed appearance; (*b*) the green parenchyma, comparing it with the pith; (*c*) the manner in which the medullary rays merge with the cells of the bark.

C. *Cell structure of the wood.* Use the cross sections employed above and also radial (lengthwise) sections and tangential (lengthwise) sections, staining if desired (Sec. 212).

1. In radial sections note (*a*) the long, empty *wood cells*, *tracheids*, with walls bearing *bordered pits* (pits characterized by two circles and peculiar to certain groups of gymnosperms); (*b*) the medullary rays, like long knife blades, piercing the wood, mostly composed of cells with dense protoplasmic contents but some of them empty and pitted. Show these points in a detailed figure.
2. In tangential sections note (*a*) the wood cells, tracheids, with cross sections of the bordered pits; (*b*) the cross sections of the medullary rays. Illustrate in a detailed figure.
3. Examine the cross sections again to understand clearly the appearance of the pits and medullary rays in the light of your study of radial and tangential sections. Make a new figure if the study outlined in B, 2, is not satisfactory.
4. Draw a cross section of a pit, showing the delicate membrane, or primitive cell wall (middle lamella), which crosses it, and the secondary thickening of the cell wall on both sides.
5. Construct a figure of the appearance of a cube of wood under high magnification, several cells wide, as viewed from an angle so as to show cross, radial, and tangential sections (App. 21). Include in this figure also one or more medullary rays and a resin duct.

D. *The cell structure, or histology, of the pine needle.* Cut cross sections of a pine needle free-hand (Sec. 194) or use prepared slides (Sec. 212). Observe:

1. The heavy *epidermis*, with lengthwise grooves, in which are situated the *stomata*.

2. The layer of *rigid tissue* (sclerenchyma) beneath the epidermis.
3. The broad layer of green tissue, *mesophyll*, whose cells have infolded walls.
4. *Resin ducts* in the green tissue.
5. A central area containing in most species of pine two *fibro-vascular bundles* and bounded by a *bundle sheath*. The fibro-vascular bundles lie in a special region of so-called *transfusion tissue*, composed in part of empty pitted cells and in part of cells containing protoplasm. Each bundle consists of a region of wood (xylem) and bast (phloëm) and contains rather ill-defined medullary rays.
6. The sections of the stomata show epidermal cells on either side of the groove and below them two small *guard cells* containing chlorophyll. Each stoma opens into a chamber within the green tissue.

Show the position of the tissues of the needle in an outline drawing and then treat the details in separate figures.

E. *The staminate cones.* These are short-lived structures developed in the late spring with the appearance of the new growth from the terminal buds. They are variously clustered in different species of pine. Draw a habit sketch of a group, showing the arrangement of the cones on the new growth, with its developing needles.

1. Observe the position of the *staminate cone* in the axil of a pointed *scale leaf*. Draw in side view to show the somewhat spiral arrangement of the closely crowded *stamens* (microsporophylls).
2. Split the cone lengthwise and diagram the attachment of the stamens along its axis. What is the morphology of the staminate cone as indicated by its position on the stem and the nature of the stamens (see F below)?

F. *The stamen and pollen.* Remove a stamen from a cone which has not yet shed its pollen.

1. Observe its form and structure, — a short stalk, broadening beyond, on the lower face of which are borne long *pollen sacs* (microsporangia). The tip of the stamen is turned upwards and fits over the pollen sacs of the stamen above. Draw under a hand lens the stamen in end and side views to illustrate these points.

2. Open a pollen sac and mount the *pollen grains* (microspores) in water. Under h.p. note (*a*) the two *wings* attached to the pollen grain; these are developed from the outer wall of the cell; (*b*) within the pollen grain the *tube nucleus* lying near the center and the *generative cell* against the wall at the side farthest away from the wings, also occasional remains of a *prothallial cell* between the generative cell and the wall. Draw a pollen grain showing this structure.

The pollen grains are developed in groups of four, *tetrads*, within *pollen mother cells*. Their method of formation shows them to be *microspores*, and the pollen sac is consequently a *microsporangium* and the stamen a *microsporophyll*. The nuclear and cell divisions within the pollen grain are stages in the germination of this microspore to form the *male gametophyte*.

G. *The carpellate cone at the time of pollination and its scales.* These cones appear on the new growth from the terminal buds in the late spring at the same time as the staminate cones. They are borne singly or in groups of two or three at the tips of branches. Draw a habit sketch of the carpellate cones on the new growth.

1. Draw a side view of the carpellate cone, showing the spiral arrangement of the *cone scales*. Each scale is believed to be a group of fused *carpels* or *megasporophylls*.
2. Detach a cone scale carefully and draw it viewed from the inner face. Note (*a*) the two *ovules* at either side of its base, each with two horn-like *appendages;* (*b*) a *point* on the cone scale above the ovules and between them.
3. Draw a side view of the cone scale, noting a small *bract* in the axil of which the cone scale is borne.

The ovule is a *megasporangium* with a protective envelope, the *integument*, but the evidence for this conclusion can only be understood after the study of sections of later stages (see J).

H. *The year-old carpellate cone and its scales.* Search for year-old cones, establishing their identity by their position on

the branches. Compare their size and texture with the carpellate cones at the time of pollination. Draw a side view of the cone, if time permits, and then detach a scale and draw two views as described in G, 2, 3, noting and comparing the relative position of the structures described there.

I. *The two-year-old carpellate cone and its scales.* Search for two-year-old cones, establishing their identity by their position on the branches. Compare their size and texture with the year-old cones and the cones at the time of pollination.

1. Draw a side view, if time permits.
2. Cut into the cone with a heavy knife, carefully detaching one of the scales. Draw the scale as viewed from the inner face, noting (*a*) that the ovules are ripening into *winged seeds*, the wings developing from a tissue that separates from the inner face of the scale; (*b*) the relative position of the *point*, above and between the maturing seeds.
3. Draw a side view of the cone scale to show the position of the bract in the axil of which the cone scale is borne and the relation of parts in comparison with the cone scale at the time of pollination.

J. *The ovule on the year-old cone.* Sections of the ovule on the scales from a year-old cone may be cut free-hand, but stained microtome sections are much more satisfactory (Sec. 212). They should be cut lengthwise of the ovule and perpendicular to the surface of the scale. Observe:

1. The enveloping *integument* meeting at the tip of the ovule where there was formerly an opening, the *micropyle*, at the time of pollination.
2. Within the integument and below the micropyle the *pollen chamber* in which germinated *pollen grains* may be found sending their *tubes* into the interior of the ovule.
3. A conical structure, the *nucellus*, into which the pollen tubes have grown, lying within the integument.
4. A large area in the interior of the nucellus, called the *embryo sac*, which contains a delicate tissue, *endosperm*,

and at the micropylar end several reduced *archegonia* with very large *eggs*.

Show in an outline drawing the relations of the structures described above, treating such details as are possible in separate figures.

The endosperm, with the archegonia and eggs, constitutes the *female gametophyte*, derived from a *megaspore* which became the embryo sac. The megaspore was one of a group of four cells, *tetrad*, formed in the interior of the nucellus, which is consequently a *megasporangium*. The integument is a special protective envelope, possibly comparable to an indusium. The pollen tubes are later developments of the male gametophytes formed by the germination of the microspores or pollen grains.

The morphology of the cone scale has been and is still a difficult problem. Because of the arrangement of the fibro-vascular bundles in the scale there are reasons for believing it to be a group of fused megasporophylls or carpels, probably two fertile carpels, each bearing an ovule, and possibly a third sterile carpel represented by the point on the scale, situated above and between the ovules. The conė scale is therefore much more complex than the stamen. According to this theory the cone scale is a group of megasporophylls in the axil of a bract, and the carpellate cone is not a simple cluster of sporophylls arranged along a shoot (like the staminate cone), but is a compound structure consisting of groups of sporophylls in the axils of bracts. The staminate cone has the same morphology as the cones of club mosses and some simple flowers of angiosperms, but the carpellate cone is comparable to an inflorescence, or cluster of flowers, each cone scale representing a highly modified flower.

K. *The gametophytes.* A full study of the gametophytes of the pine would require the examination of stages in material covering many months of development, which is impracticable in a general course. In slides of the stage treated in J it will be possible to determine the following structures :

1. In the female gametophyte : (*a*) the delicate cell structure of the *endosperm ;* (*b*) the protoplasmic structure of the large *egg* with its

prominent nucleus; (*c*) frequently the presence of a single *canal cell* (ventral canal cell) above the egg; (*d*) a layer of cells differentiated from the endosperm, forming a *jacket* around the egg; (*e*) one or two tiers of cells, four cells in a tier, above the egg, and representing the neck of the much-reduced archegonium (see *Principles*, Fig. 300, *D*).

2. In the male gametophyte: should the tips of pollen tubes be found entering the necks of archegonia they may be expected to show two large *sperm nuclei*, and possibly the remains of the *tube nucleus*, now degenerating, with that of the *stalk cell* also.

L. *The seed.* Take seeds from an open cone. It generally opens at the end of the third summer, when the cone is approximately two years and three months old. Sketch to show the wings. Section such seeds, or, better still, cut open some of the large edible seeds of the nut pines, piñon, obtainable from fruit dealers. Note:

1. The tough seed coat, or *testa*, which is the ripened integument, and beneath the testa a membranous seed coat which is largely the remains of the *nucellus*.
2. The *endosperm*, filling the seed except for the *embryo*. The former is a development from the endosperm of the embryo sac and consequently gametophytic in character.
3. The straight *embryo*, developed from a *fertilized egg*, attached to the micropylar end of the seed by a *suspensor* and imbedded in the endosperm. The embryo consists of a short *hypocotyl*, bearing above a circle of *cotyledons*.

Construct a diagram of a lengthwise section of a seed to show these structures in relation to one another.

M. *The germination of the seed.* The pine seed germinates rather slowly, but it will be of interest to plant some and watch them as they sprout, comparing them with such seedlings of the angiosperms (squash, bean, pea, corn, etc.) as may have been studied.

Reference. *Principles*, Secs. 350–356.

Questions. What are the growth habits of the pine? What are the peculiarities of its foliage? its adaptation to drought

and cold? Where is the resin and turpentine formed? Can you suggest any advantage to the plant in its production? How and when is pollen formed and how abundantly? How does it reach the ovule? What is the history of the carpellate cone after pollination? When are the seeds ripened? What is the morphology of the pollen grain? Describe the male gametophyte, with its habits, after the germination of the pollen grain in the pollen chamber. Describe the structure of the ovule. Describe the female gametophyte and its life habits within the nucellus (megasporangium). From what does the embryo arise and how does it obtain the food for its development? How many generations are represented in the tissues of the seed? Describe the life history, distinguishing between the sexual phases, gametophytes, and the asexual phase, sporophyte. Draw and arrange a series of diagrams illustrating the chief stages throughout the life history, using two colored pencils to designate the gametophytic and sporophytic generations respectively (App. 18). Construct a life-history formula that will express this succession (App. 18).

139. The morphology of the juniper and arbor vitæ. The juniper (*Juniperus*) and arbor vitæ (*Thuya*) are interesting types to study comparatively with the pine: (1) as regards the arrangement and forms of the leaves and consequent appearance of the foliage; (2) as regards the structure of the carpellate cones, whose scales are opposite instead of being distributed spirally, and present other peculiarities of structure and habits of ripening; (3) with reference to the special characteristics of the stamens. These genera present a higher type of gymnosperm evolution in these respects than the pine. Certain cedars (*Cupressus*) are equally good for this comparative study.

THE ANGIOSPERMS, OR ANGIOSPERMÆ

140. The morphology of the angiosperms. The general morphology of the angiosperms, including roots, stems, leaves, flowers, and fruits, together with many principles of plant physiology, have been treated in Part I, The Structure and Physiology of

Seed Plants. The outlines presented here will deal entirely with the gametophyte generations and the organs of the sporophyte, stamens and pistil (composed of carpels), especially concerned with their development. For outlines covering general flower structure see Secs. 44–46. An outline for a general type study of an angiosperm such as the lily is presented in Sec. 162.

141. The lily studied with reference to its gametophytes (App. 22). The lily is a favorite subject for the study of pollen formation and the development and fertilization of the embryo sac, partly for its clearness and partly for the relative ease with which material may be obtained. Other types of the lily family, such as the trillium, the tulip, the Roman hyacinth, etc., are also good. Satisfactory studies on the gametophytes of the angiosperms require microtome sections of the structures involved. Directions for the preparation of these are outlined in Sec. 212. The wild lilies, such as *Lilium philadelphicum*, furnish excellent material, but various cultivated lilies are equally good or better.

A. *The stamen of the lily.* Dissect away the perianth of the lily flower to show the stamens and pistil.

1. Observe the arrangement of the *stamens* around the *pistil.* Draw a stamen to show the *stalk* (filament) and the attachment of the *anther.*
2. Note how the pollen is discharged from the anther.
3. Draw a pollen grain under h.p. to show the markings on its wall.
4. Section the anthers and observe that the pollen is developed in four *locules*, or *pollen sacs*, running lengthwise of the anther.
5. In microtome sections properly stained (Sec. 212) note that the pollen grains will show a large central nucleus, *tube nucleus*, and at one end the *generative nucleus*, which gives rise later to two *sperm nuclei.*

B. *The development of pollen.* To obtain the stages of pollen formation anthers must be taken from very young unopened buds, the stamens of which when cut across exude a watery fluid from the pollen sacs. Should the fluid be milky or yellowish the stamen is too old and will certainly contain pollen grains. Stamens of the proper age must be fixed, imbedded, and cut lengthwise on the microtome and stained as described in Sec. 199, D. Such preparations will show various stages in development and division of the pollen mother cells to form the pollen grains in groups of four, or *tetrads.* The following conspicuous stages are likely to be found and should be drawn.

1. The *spore mother cells* before division, with their nuclei in a *resting condition.* They constitute a *spore-forming tissue* (archesporium),

and, increasing in size, gradually round off and separate from one another.

2. *Synapsis*, a very common stage in which the *chromatin* within the nucleus of the spore mother cell will be found collected in a dense mass, generally near the *nucleolus* at one side of the nucleus. Synapsis is a very important stage, since it appears to be characteristic of the time when the sporophyte number of chromosomes is *reduced* by half to the number of the gametophytes (see *Principles*, Sec. 334). The gametophyte number of chromosomes in the lily is twelve, and first appears in the nuclear divisions within the pollen mother cell and the embryo sac (D, 3).
3. The *first nuclear division*, or *mitosis*, where a large spindle will be found within the spore mother cell, and the chromosomes will be either at the center, forming the *equatorial plate* (see *Principles*, Fig. 302, *B*), or separated into two groups of *daughter chromosomes* that pass to the poles of the spindle.
4. *Two daughter nuclei* in a resting condition following the first mitosis and the division of the spore mother cell into *two daughter cells.*
5. The *second nuclear division*, or mitosis, in which two spindles are formed simultaneously in the two daughter cells, resulting in *four daughter nuclei.*
6. The final division of the spore mother cell into *four daughter cells*, forming a *tetrad*, each of which becomes a *pollen grain.*

The processes of pollen formation are identical in all essentials with those of spore formation in the bryophytes and pteridophytes, and show that the pollen grains are *microspores*, — a conclusion sustained by their further development into reduced *male gametophytes.* The pollen sac is therefore a *microsporangium* and the stamen a *microsporophyll.* Pollen formation in the lily and related types is an attractive subject for the study of nuclear and cell division in the higher plants.

C. *The pistil of the lily.* Draw the pistil in side view, showing :

1. The *ovule case*, or *ovary*, below ; the *style* above, bearing the three-lobed *stigma*, or receptive region, for the pollen. Note that the ovule case is three-angled, and the position of the angles with reference to the lobes of the stigma.
2. Cut sections of the ovule case both from flowers which have recently opened and from those whose perianth has been withered several days. Under l.p. note the three *loculcs*, or *chambers*, of the ovule case and the *ovules* within them. Show their position in an outline drawing.

The three locules of the ovule case and the three lobes of the stigma present evidence that three *carpels* are involved in the formation of this

pistil. That the carpels are *megasporophylls* is proved by the structure and development of the ovules (see D).

D. *The development of the ovule and embryo sac.* These can best be studied from microtome cross sections of the ovule cases prepared as described in Sec. 212. The ovule cases from open flowers contain mature embryo sacs. Those collected from three to four days after pollination may show stages in fertilization (the time varies in different lilies). Stages in the development of the embryo sac must be sought in unopened buds along with or somewhat later than stages in pollen formation. The following stages are likely to be found in material of graduated ages and should be drawn:

1. Young ovules with the two *integuments* beginning to develop around the *nucellus*. The end of the nucellus will be exposed, and at the tip, under a layer of cells, is to be found a large cell with deeply staining protoplasmic contents and conspicuous nucleus. This becomes the *embryo sac*.
2. Later stages show the inner integument grown beyond the nucellus and forming the *micropyle* at the tip of the ovule. The outer integument extends around on the outside nearly to the end of the inner integument.
3. The embryo sac, gradually enlarging with the growth of the ovule, is the seat of *three nuclear divisions*, or *mitoses*, by which the number of nuclei is increased to eight. The gametophyte number of chromosomes, twelve, appears in the first of these mitoses as in the pollen mother cell (B, 2). The first two of these mitoses have peculiarities (see *Principles*, Sec. 360 and footnote) which show that they are of the same kind as those characteristic of *pollen formation* and *spore formation* when *tetrads* are developed within *mother cells*. But tetrads are no longer developed in the nucellus of the lily, although present in the ovules of many other angiosperms (see *Principles*, Fig. 304). The four nuclei resulting from the first two mitoses in the embryo sac of the lily, although comparable to megaspore nuclei, have all become included in the very much reduced female gametophyte that is developed in the embryo sac.
4. The eight nuclei of the mature embryo sac become distributed as follows: (*a*) three nuclei form the *egg apparatus* at the micropylar end of the sac, one being the *egg nucleus* and lying slightly below and between the other two, which are termed *synergids;* (*b*) three nuclei form the group of *antipodal nuclei* at the opposite end of the sac; (*c*) the other two, called *polar nuclei*, approach one another in the center of the sac. This is the structure of the mature female gametophyte (see *Principles*, Fig. 306, *B*).

The first two mitoses in the embryo sac of the lily show that it is a *megaspore mother cell* in this plant (as also in related types), which later contains the *female gametophyte*. The nucellus is therefore a *megasporangium* and the carpel a *megasporophyll*.

E. *Fertilization and double fertilization.* Stages showing the nuclear fusions of fertilization and double fertilization are, of course, not common, but one preparation will serve for a demonstration of the processes. The pollen tube brings two *sperm nuclei* into the embryo sac. One of these unites with the *egg nucleus*, fertilizing it, and the other unites with the *two polar nuclei*, forming a triple fusion which results in the large *endosperm nucleus* in the center of the sac (see *Principles*, Fig. 307).

F. *The development of the embryo and endosperm.* The fertilized egg nucleus with surrounding protoplasm becomes the *fertilized egg*, and, forming a cell wall about itself, proceeds to develop the *embryo sporophyte* at the micropylar end of the embryo sac. The endosperm nucleus gives rise through successive divisions to a very large number of nuclei, which become distributed in the layer of protoplasm which lines the embryo sac (see *Principles*, Fig. 308). Cell walls are later formed between these nuclei, and the embryo sac becomes filled with a delicate tissue called the *endosperm*, in which the developing embryo lies imbedded.

It is important to note that the endosperm of all the angiosperms is a development following fertilization and therefore not strictly comparable to the endosperm of gymnosperms, as illustrated in the pine, which is formed before fertilization and is therefore strictly gametophytic in character without the complication of double fertilization on the union of polar nuclei. The group of three antipodal nuclei in the embryo sac of the angiosperms may represent the endosperm of gymnosperms, but this is not fully established. The female gametophyte of the angiosperms is much simpler than that of the gymnosperms, having only eight nuclei, one egg, and no clearly defined archegonium. The male gametophyte of the angiosperms is likewise simpler, containing only three nuclei, two sperm nuclei, and the tube nucleus.

142. The pollen grain, or microspore of the elder. Some pollen grains at maturity contain the male gametophytes much further advanced than is shown in those of the lily. This is well illustrated in the elder (*Sambucus*). Unopened stamens should be fixed and preserved in alcohol. The anthers of such may be teased apart in water, allowing the pollen grains to escape. When stained with eosin these grains will be seen to contain three nuclei, a *tube nucleus* lying freely in the center of the cell, and two *sperm nuclei* somewhat at one side, surrounded by denser protoplasm, forming two male cells (see *Principles*, Fig. 305). The nuclear divisions are completed in this male gametophyte at the time the pollen is shed, and the only further

development is that of the pollen tube, which carries the sperm nuclei to the embryo sac. Microtome sections of the ripe anthers of the elder (Sec. 212) will give excellent preparations of this interesting condition.

The pollen grains of many plants, as the lily, contain but two nuclei at the time of pollination. These are the tube nucleus, above mentioned, and the generative nucleus, which later gives rise to the two sperm nuclei.

143. Capsella, the shepherd's purse, studied for the development of the flower, ovule, pollen tube, and embryo (App. 23). The shepherd's purse is in many respects an excellent type for a general study of a dicotyledonous seed plant, although the flower is rather small. It is a particularly good subject for the study of the topics indicated in the above heading.

A. *The development of the flower.* Tease apart in a drop of water, under a dissecting microscope, the extreme tip of a flower cluster (which is a raceme), to obtain the youngest flowers (microscopic) just below the growing point. Older stages may be cleared by adding potash solution if necessary (Sec. 169). Search for the following stages and draw:

1. The young flowers appearing as small protuberances just back of the growing point.
2. A circle of *sepals* developing at the tip of the young flower.
3. The growth of the sepals over the tip of the flower and the origin of the *stamens* in a circle within.
4. The development of two *carpels*, more or less united below, at the tip of the flower.
5. The late appearance of the *petals* between the sepals and stamens, after all the other parts of the flower are present.
6. The final folding one over the other of the sepals in the young bud, the growth of the petals, the differentiation of the stamens into *anthers* and *stalks* (filaments), the union of the carpels above to form the *pistil*, with the *ovule case* or *ovary* below, in which the *ovules* appear as outgrowths along the surface.
7. Microtome sections cut lengthwise of the tip of the raceme (Sec. 212) give excellent stages in the development of the flowers, and especially the closing together of the carpels to form the pistil and the origin of the ovules along their inner surface within the ovule case.

B. *The development of the ovule.* Pick to pieces some of the youngest flowers that can be easily seen with the unaided eye. Open the ovule cases, or ovaries, in a drop of water, with the point of a needle, thus exposing the ovules. A variety of stages will be presented. Search for the following and draw:

1. The young ovule, consisting of the *nucellus* at the end of a short stalk, with the *two integuments* just beginning to appear like collars at its base.

2. A stage in which the integuments are further developed, the outer arising somewhat below the inner one. The large *embryo sac* is generally evident at this time in the center of the nucellus.
3. A stage in which the outer integument has grown over and beyond the inner one, so that only the extreme tip of the nucellus is seen. At this time the ovule begins to bend over at its basal region.
4. Finally the outer and inner integuments grow completely over the nucellus, almost meeting beyond to form the small opening called the *micropyle*. Meantime the bending of the ovule brings the micropyle close to the stalk of the ovule, so that the latter is therefore completely bent on itself (campylotropous). This condition will be found in the ovules of rather young, unopened flowers, and such preparations may be cleared with potash.

C. *The development of the pollen tubes.* Mount the pistil of an open flower in water and examine the *stigma* under m.p. Note the *papillæ* on its surface, and among the papillæ the germinating *pollen grains*, which will be found sending *tubes* into the tissue of the stigma. Clear with potash if too opaque. Draw.

D. *The development of the embryo.* Remove the *pistils* from flowers, the petals of which have begun to wither. Open the ovule case with a needle and mount the ovules in a potash solution. The *embryo* may sometimes be clearly seen lying in the *embryo sac.* Press gently on the cover glass and the embryo will be crushed or squeezed out. Note the row of cells forming the *suspensor*, the lower one of which is much enlarged. Make a number of preparations of younger and older ovules, which are likely to show the following stages, and should be drawn :

1. The *suspensor* before the formation of the embryo, consisting of a filament attached by a large basal cell near the micropylar end of the embryo sac.
2. The development of the *embryo*, beginning at the free end of the suspensor, by the formation of walls in three planes, thus differentiating a globular structure.
3. The later growth of the embryo, with the appearance of two *cotyledons*, and the development of the *root* at the point where the embryo is attached to the suspensor.
4. Lengthwise microtome sections of the ovules (Sec. 212) will show the relation of the developing embryo to the enlarged and curved embryo sac with its *endosperm*, and the *integuments* of the ovule (see ***Principles***, Fig. 309, *H*).

Part II of this manual presents a series of type studies furnishing an outline of the comparative morphology and life histories of plants, upon which are based systems of classification and various theories of the evolution of the groups. While botanists are in general agreement on the principal lines of plant evolution and in full accord as to the fact that there has been an evolution, the details of the history of development must always remain speculative problems for the reason that evolutionary processes have been in force since very early geological ages and the records of plant life in former periods, preserved as fossil remains, are relatively scanty. Consequently, while it is often of great interest to draw or diagram lines of plant evolution indicating relationships of groups, such outlines should generally be considered as provisional attempts to help forward discussion rather than as expressions of final judgment.

Such a study of comparative morphology as may be framed from the matter presented in Parts I and II forms an excellent foundation for detailed work in plant physiology and the fundamentals of ecology. Indeed, it may be said to be essential to extended work in these subjects, for a knowledge of structure must always precede a study of functions and life activities.

When a course in botany is planned to begin with type studies such as may be chosen from Part II, the usual procedure would be to supplement or end the course with the more special examination of the seed plants. These latter studies may be not only morphological and physiological but also ecological, and would involve a selection of such topics and experiments from Parts I and III as seem best suited to the conditions under which the work must be given.

Part III

ECOLOGY

PARASITIC AND CARNIVOROUS PLANTS

144. Field study of parasites.

A. Study out of doors any parasitic seed plants that you can find. In most parts of the country the dodders are of much more frequent occurrence than any other parasites among the higher plants. Frequently several species of dodder can be found.

Note and collect the host plants on which the parasite grows and observe the mode of attachment between parasite and host. Find out whether the host is at all injured by the parasite.

B. If possible transplant thriving specimens of the parasite upon new kinds of host and see whether they will grow there. Collect seeds of any parasitic plants found, germinate the seeds, and make studies of the behavior of their seedlings. Are the seedlings green at first?

Reference. Kerner-Oliver, 2.

145. Field study of carnivorous plants.

A. Examine any carnivorous plant which you can find growing spontaneously (various species of *Drosera* and *Sarracenia* are the kinds most widely distributed). Make notes on the total number of insects captured by a single plant and by a single leaf.

B. In the case of *Drosera* study and draw various leaves, some expanded and some closed over insects.

C. Put into 50 per cent alcohol or 2–4 per cent formalin solution all the insects obtained from any one kind of carnivorous plant and bring them to the laboratory for determination of the groups.

146. Laboratory study of carnivorous plants.

A. Put living flies in the pitchers of *Sarracenia* containing their usual amount of liquid, and note whether any of the flies escape, or what finally becomes of them.

B. Feed leaves of *Drosera* with bits of raw meat, particles of cheese, very small insects, bits of sand, or broken glass. Place the objects very

carefully on the tips of the glandular hairs, note what movements, if any, occur, make sketches of the leaf in various positions, and keep a complete daily record of the behavior of the leaf until it returns to its ordinary form.

REFERENCES for Secs. 145, 146. Darwin, 64; Darwin and Acton, 11.

HOW PLANTS PROTECT THEMSELVES FROM ANIMALS

147. Field study. If possible, visit pastures grazed by horses, cattle, or sheep, and large barnyards where weeds are abundant. Make a sketch map of a small part of the pasture or barnyard, showing the clumps of weeds that have been left uneaten; number the clumps, and at the bottom of the map indicate what plants make up each group. Study the characteristics of some plants that are not usually eaten, and state the most obvious means of protection of each plant.

148. Laboratory study. Examine in detail any of the plants of your region which are left unharmed by grazing animals, and make out a tabular list of the protective equipment of each plant. Use the microscope to study and make sketches of cutting edges of grasses and sedges and the rough or stinging hairs or spines of such plants as mulleins, nettles, thistles, and so on. Do not taste plants suspected of being poisonous, but try those which are known not to be so. If some plants are attacked by insects, though not by grazing quadrupeds, make a note of it. Record the notes in a table like the one on the following page.[1]

POLLINATION OF FLOWERS

149. Studies in insect pollination.** The student cannot gather more than a very imperfect knowledge of the details of cross pollination in flowers without actually watching some of them as they grow and observing their insect visitors. If the latter are caught and dropped into a wide-mouthed, stoppered bottle containing a bit of cotton saturated with chloroform, most of them may be identified by any one who is familiar with our common insects. The insects may be observed and classified in a general way into butterflies, moths, bees, flies, wasps, and beetles, without being captured or molested.

Whether these out-of-door studies are made or not, several flowers should be carefully examined and described as regards their arrangements for attracting and utilizing insect or bird visitors.

[1] It will probably be necessary for the instructor to determine for the student many of the species studied.

MEANS OF PROTECTION OF CERTAIN PLANTS

	Poisonous	Of disagreeable flavor	Of unpleasant scent	Spiny	Rough, hairy	With stinging hairs	With cutting leaves	Tough or woody	With other protection
Jimson weed (*Datura*)	×	?	×	×					

REFERENCES. Kerner-Oliver, 2; Ludwig, 51.

OUTLINE FOR STUDY OF ADAPTATIONS FOR POLLINATION[1]

I. Is the flower characterized by
- 1, greenish, nectarless, inconspicuous perianth?
- 2, appearing before the leaves?
- 3, dry, dusty pollen?
- 4, feathery stigmas?

If several of these characteristics are found, it is probably *wind-pollinated.*

II. Is the flower characterized by
- 5, curving stamens which bring the anthers into contact with the stigma? If so, it is *self-pollinated* (see *Principles*, Fig. 330).[2]

[1] Pollination by water is not discussed here, as the cases are rather few and material is not usually available for study.

[2] Many self-pollinated flowers are not easily distinguished as such.

III. Is the flower characterized by
- 6, odor?
- 7, color (not green)?
- 8, nectar?
- 9, sticky pollen?
- 10, opening during only part of the day?
- 11, bilateral symmetry?
- 12, facilities for insect visitors? (See *Principles*, Figs. 324, 325.)[1]
- 13, mechanism for holding visitors imprisoned until covered with pollen?
- 14, mechanism for pollinating visitors? (See *Principles*, Figs. 331, 332.)[1]
- 15, two or three lengths of stamens and pistils (dimorphism or trimorphism)?
- 16, unequal maturing of stamens and pistil (*dichogamy*)?

If any of these characteristics (III) are found, the flower is pollinated by insects, birds, or other animals.

Make a list of all the attractions displayed by the flower examined, and if possible find out what visitors it receives and how their visits are utilized.

Is the flower under examination protected from undesirable visitors by means of
- 17, a sticky stem or flower stalk?
- 18, water reservoirs along the stem?
- 19, a slippery flower stalk?
- 20, a sticky-hairy or slippery nodding calyx?
- 21, a corolla with closed throat?
- 22, stamens or pistils covering the nectaries?
- 23, a long calyx or corolla tube?
- 24, long spurs, with the nectar stored at the bottom?

Make a list of these protections, and if possible study their operation.

The following list includes a considerable number of the most accessible flowers of spring and early summer, about which it is easy to get information from books.

List of Insect-Pollinated Flowers[2]

I

1. Flax . . . . . *Linum usitatissimum* . . . . . Knuth
2. Missouri currant . . . *Ribes aureum* . . . . . Knuth

[1] For very many other devices for pollination see Knuth-Davis, 62, and Kerner-Oliver, 2.

[2] The plants in this list are arranged somewhat in the order of the complexity of their adaptations for insect pollination, the simplest first. It would be well for

3.	Snowberry	*Symphoricarpus racemosus*	Knuth
4.	Lilac	*Syringa persica*	Knuth
5.	Periwinkle	*Vinca minor*	Knuth
6.	Mignonette	*Reseda odorata*	Knuth
7.	Lily of the valley	*Convallaria majalis*	Knuth
8.	Dead nettle	*Lamium album*	Lubbock
9.	Bleeding heart	*Dicentra* (*Diclytra*) *spectabilis*	Knuth
10.	Columbine	*Aquilegia vulgaris*	Knuth
11.	Monkshood	*Aconitum Napellus*	Knuth

II

12.	Larkspur	*Delphinium elatum*, *D. consolida*	Knuth
13.	Herb Robert	*Geranium robertianum*	Knuth
14.	Pink	*Dianthus* (various species)	Knuth
15.	Fireweed	*Epilobium angustifolium*	Gray
16.	"Nasturtium"	*Tropæolum majus*	Newell, Lubbock
17.	Pansy	*Viola tricolor*	Knuth
18.	Heal-all	*Brunella* (*Prunella*) *vulgaris*	Knuth
19.	Ground ivy	*Nepeta Glechoma*	Knuth, Newell
20.	Lousewort	*Pedicularis canadensis*	Knuth, Newell
21.	Snapdragon	*Antirrhinum majus*	Knuth
22.	Iris	*Iris versicolor*	Newell
23.	Bellflower	*Campanula rapunculoides*	Knuth
24.	Horse-chestnut	*Æsculus Hippocastanum*	Newell

III

25.	Yarrow	*Achillea millefolium*	Knuth
26.	Oxeye daisy	*Chrysanthemum Leucanthemum*	Knuth
27.	Dandelion	*Taraxacum officinale*	Knuth, Newell

IV

28.	Barberry	*Berberis vulgaris*	Lubbock
29.	Mountain laurel	*Kalmia latifolia*	Gray

each student to take up the study of the arrangements for the utilization of insect visitors in several of the groups above, numbered with Roman numerals. Explanations of the adaptations can be found in the works cited by abbreviations at the right. Knuth stands for Knuth-Davis's *Handbook of Flower Pollination*, 62; Lubbock, for *British Wild Flowers, considered in Relation to Insects*; Gray, for Gray's *Structural Botany*; and Newell, for Miss Newell's *Outlines of Lessons in Botany*, Part II. Consult also Weed's *Ten New England Blossoms*, and Kerner-Oliver, 2. The instructor may find it necessary to identify most of the species.

V

30. White clover	*Trifolium repens*	Knuth
31. Red clover	*Trifolium pratense*	Knuth
32. Locust	*Robinia Pseudacacia*	Gray
33. Wistaria	*Wistaria sinensis*	Gray
34. Vetch	*Vicia Cracca*	Knuth
35. Pea	*Pisum sativum*	Knuth
36. Bean	*Phaseolus vulgaris*	Gray
37. Groundnut	*Apios tuberosa*	Gray

VI

38. Partridge berry	*Mitchella repens*	Gray
39. Primrose	*Primula grandiflora*, *P. officinalis*	Lubbock
40. Loosestrife	*Lythrum Salicaria*	Gray

VII

41. Milkweed	*Asclepias Syriaca*	Knuth, Newell

VIII

42. Lady's slipper	*Cypripedium acaule*	Newell

HOW PLANTS ARE SCATTERED AND PROPAGATED

150. Field study of vegetative propagation.

A. Collect, by digging them up, sketch, and describe any underground stems of use in multiplying plants. There are many rootstock-producing species, such as June grass, quick grass, Bermuda grass, Canada thistle, common sorrel (*Rumex Acetosella*), wild iris, wild ginger, sweet flag, various sunflowers, and some mints. If possible dig away the earth from one side of a hill of potatoes, and sketch some of the roots, subterranean branches, and tubers. Bulbs are borne by a large proportion of the members of the lily family.

B. Make sketches to illustrate the spread of plants by rooting branches, as in the raspberry, strawberry, and cinquefoils.

C. In any cleared or partially cleared bit of woodland note the way in which some trees produce a crop of sprouts from the stump.

D. Examine the neighborhood of black locust trees (*Robinia*), silver-leaved poplars, or balm of Gilead poplars, to find out how far these trees "spread by the root." Dig up a young sprout, with a piece of the parent root, and sketch it.

Reference. Beal, 63.

151. Laboratory study of vegetative propagation.

A. In moist earth or sand plant pieces of potato tubers, each containing one or more "eyes," and others without eyes. Note results and sketch any plants that are produced.

B. Plant bulbs and bulblets of onion and note the comparative growth of the plants produced.

C. In sand which is kept moist plant cuttings of any of the following plants: "geranium," *Tradescantia*, willow, cottonwood currant, raspberry, blackberry, and grapevine. When the cuttings have rooted, sketch some of them, and decide whether the roots spring indifferently from any part of the stem.

D. If obtainable, put some vigorous *Bryophyllum* leaves on moist sand, cover with a bell glass, and sketch the leaf with young plants, if any appear.

152. Field study of dissemination of seeds.

A. Examine the region about any tree or shrub which has no near neighbors of its own kind, and try to trace the distance to which its seeds have been carried. In case enough seedlings are found to warrant it, make a map to show their distribution with reference to the parent tree.

B. Discuss the means by which the seeds have been carried.

C. Watch such trees as elms, maples, lindens, willows, sycamores, and cottonwoods, when the fruits or seeds are fully ripe, and find out how far they travel. What trees hold many of their seeds after they are fully ripe? What are the advantages of this?

D. Look for as many contrivances for seed dispersal as possible, and classify the plants studied into those with fruits or seeds dispersed:

(1) by wind.
(2) by water.
(3) by animals.
(4) by some contrivance for shooting or slinging the seeds.

Reference. Kerner-Oliver, 2.

153. Laboratory study of dissemination of seeds.

A. Find out which of the wind-carried fruits and seeds fall most slowly from the laboratory ceiling to the floor.

B. Test some of the following water-carried fruits and seeds to see which will float longest: aquatic grasses, rushes, and sedges, polygonums, water dock, bur reed, arrowhead, water plantain, pickerel weed, alder, buttonbush, water parsnip (*Sium*), water hemlock (*Cicuta*), water pennywort (*Hydrocotyle*), lotus (*Nelumbo*).

C. Sketch and describe in detail the fruit or seed which appears to be best adapted to each of the four modes of dispersal above mentioned (Sec. 152, D).

COMPETITION AND INVASION

154. Field study of competition.

A. Find a spot in which many weed seedlings have sprung up, stake off one or two square feet, count the plants, and then watch their growth for as long a period as possible. Stake off a similar plot, pull up all but one or two of the weeds, and compare the growth of these plants with that of the crowded ones.

B. Beginning as soon as weed seedlings start in the spring, stake off a square foot of very weedy ground, pull up and count all the seedlings which grow on the plot, continue the count as others spring up, and make a list of the kinds obtained [1] and the total number of each kind.

C. Allow any large, vigorous weeds to grow up among lettuce, radish, carrot, or other seedlings, and notice which set of plants prevails. Give as many reasons as possible for this result.

References. Clements, 59; *Principles*, Chapter XXXIV.

155. Field study of invasion.**

A. Look for places in which pastures or mowing fields are beginning to "run out," and daisies (*Chrysanthemum*), sorrel (*Rumex*), cone flowers, and similar weeds are taking the place of the grass.

Look for a lawn that is too much shaded and becoming filled with chickweed or other weeds.

B. Find a pond in process of drying up and notice what changes are taking place in the character of the vegetation.

C. Study an abandoned strawberry bed.

D. Examine a clearing in which young saplings have begun to grow to a height of 15 or 20 feet. In every case make a list of the older inhabitants of the territory [1] and of the newcomers [1] and give as many reasons

[1] Identified by the instructor.

as possible for the prevalence of the latter. State what would probably be the condition of the piece of ground examined if left unmolested for ten years or more.

REFERENCES. Clements, 59; *Principles*, Chapters XXXIV, XXXV.

PLANT SUCCESSIONS

156. Field study of successions.

A. Examine a field of wheat, rye, oats, or barley, when the grain is not more than a foot high, again when it is ready for reaping, and finally as long as possible after reaping, before fall plowing or frost destroys the plants upon it. Make a list of all the plants that can be recognized at each period, and note which ones are in blossom or in fruit.

B. Study wood lots with full-grown trees upon them, others from which the trees have recently been cut off, others still which have been cleared for many years. Make a general statement of the kinds and relative numbers of seed plants of all sorts found in each case.

C. If possible, study the changes in the vegetation of old fields allowed to grow up to weeds and bushes.

Draw up a general account of what you have found to be the order of succession of plants in grain fields, in cleared woodland, and in abandoned fields. Try to give some reasons why the plants succeed one another in the order actually observed.

REFERENCES. Clements, 59; Schimper-Fisher, 56; Warming-Graebner, 57; *Principles*, Chapter XXXV.

ECOLOGICAL CLASSES

157. Field study of ecological classes.**

A. Examine the vegetation of any accessible lake, pond, marsh, or river, of ordinary woods, thickets, and grass lands, and of the driest areas in the region, such as sand hills or dunes, barren knolls or banks, ledges or outlying masses of rock. Select some of the typical inhabitants of each region and make a list of:

(1) Hydrophytes { (*a*) living only in water.
(*b*) living either in water or in very wet soil.

(2) Mesophytes.

(3) Xerophytes.

Describe the characteristics of each group, giving attention to all the vegetative parts of the plant body.

B. In woods or thickets make lists of the sun plants and shade plants, and classify the trees roughly as regards their tolerance of shade conditions. Measure the relative illumination of some of the plants which live in the deepest shade by the method given in Exp. XXVIII.

C. Study the distribution of plants as related to the character of the soil, looking for species characteristic of limestone and of sandy soils. If possible, find assemblages of plants in loose sand, especially of sand dunes, and report on their peculiar form and habits. If there are accessible localities for halophytes, examine the vegetation of salt marshes, of the sea beach, or of "alkali" lands, and describe some of the most noticeable characteristics of these plants.

D. Examine and report on any epiphytes that may be found.

References. Clements, 59; Warming-Graebner, 57; Pound and Clements, 58.

158. Laboratory study of ecological classes.** Make a sketch of at least one typical member of each of the three principal ecological classes as based on water requirements. Make careful studies of any available material and discuss as many as possible of the following topics:

A. Relative importance of the root system in aquatic plants.

B. Special provisions for photosynthesis, respiration, and circulation of air in the plant body of aquatic plants.

C. Comparison of the structure of the leaves of deciduous trees, broad-leaved evergreens (such as holly, live oak, and some rhododendrons), and needle-leaved evergreens. In this study pay especial attention to the total area of the three kinds of leaves, to their relative thickness, to the thickness of the cuticle and the epidermis, and to the protection of stomata by their position at the bottom of grooves or pits in the epidermis.

D. Appearance of plants in their resting condition (during winter's cold or summer's drought) in any available bulbous- or tuberous-rooted species or in fleshy-rooted biennials (e.g. parsnips, beets, or carrots).

E. Characteristics of plants slightly, moderately, and decidedly xerophytic, as illustrated by wild plants of the neighborhood or (if these are not obtainable) by such species as *Euphorbia splendens*, houseleek, *Echeveria*, and cactuses.

F. Difference in the appearance of a species grown in dry soil, with leaves exposed to warm, dry air, and that grown in damp soil under a

bell glass (e.g. young plants of any xerophytic grasses, houseleeks, dandelions, shepherd's purse, gorse[1]).

G. Tolerance of salt, as determined by water cultures, in one to six per cent solutions of seedlings of ordinary garden annuals and seedlings of such halophytes as the salt-marsh grasses (*Spartina* and others), marsh rosemary (*Statice*), samphire (*Salicornia*), and saltwort (*Salsola*). The plants which live longest with the roots immersed in a solution of any given strength are the most decidedly halophytic.

References. Kerner-Oliver, 2; Schimper-Fisher, 56; Warming-Graebner, 57; Haberlandt, 33.

PLANT FORMATIONS; ZONATION

159. Field study of formations.** Visit any well-defined formations that are readily accessible, such as lake or pond, marsh, river valley ("bottom land"), upland woods, hill or cliff side, wet prairie, dry prairie, and other formations.

A. Note what are the characteristic plants of each kind of formation and, if necessary, collect specimens of these and bring them to the laboratory to be named.

B. Make a detailed study of at least one formation, noting all that is possible of the physical conditions, especially of moisture and light, the habits of life, and mutual relations of the plants which compose it. For example, if the formation is a wooded one make lists of:

(1) The trees, their grouping, relative height, relative density of shade, comparative number of each species, probable origin (e.g. means by which seeds were planted and source from which they came).

(2) The larger shrubs.

(3) The undershrubs.

(4) Herbaceous plants.

(5) Parasitic or saprophytic seed plants.

If there is any law to account for the way in which the plants of (2), (3), and (4) are unequally distributed over the

[1] *Ulex.*

forest floor, try to establish it, noting especially their relation to the relative moisture of the soil and to light.

References. Clements, 59; *Principles*, Chapter XXXVII; Warming-Graebner, 57; Schimper-Fisher, 56.

Fig. 6. Model for grouping of drawings in type studies

A, base of a plant of shepherd's purse (*Capsella bursa-pastoris*), × ½; *r*, the main root; *B*, upper part of the inflorescence, × 1; *C*, two leaves, — I from the upper part; II from the base of the plant, × 1; *D*, a flower, × 3; *E*, the same, with sepals and petals removed, × 3; *F*, petal; *G*, sepal; *H*, stamen, × 10; *f*, filament; *an.*, anther; *I*, a fruit with one of the valves removed to show the seeds, × 4; *J*, longitudinal section of a seed, × 8; *K*, the embryo removed from the seed, × 8; *l*, the first leaves (cotyledons); *st.*, the stem ending in the root; *L*, cross section of the stem, × 20; *fb.*, fibro-vascular bundle; *M*, a similar section of the main root, × 15; *N*, diagram of the flower. — After Campbell

160. Field study of zonation.

A. Select any locality where two or more plant formations meet and determine:

1. The characteristic plants of each formation.

2. What plants (if any) are common to two or more formations.
3. Some of the causes of the boundaries between formations, and whether these boundaries are fixed or shifting.

B. Make a sketch map of the series of zones on the same general plan as that of Fig. 366 in *Principles*, and if possible secure one or more photographs of the series, with a large numbered placard set up in a prominent position in each zone.

STUDY OF TYPES OF SEED PLANTS *

161. Family Pinaceæ.[1]

162. Family Liliaceæ. Any obtainable genus may be used to represent the family. Lilies, tulips, dogtooth violets (*Erythronium*), *Scilla sibirica*, or Roman hyacinths answer excellently. Since it is to be had of florists during the winter months and in gardens for a long time in spring, the lily of the valley (*Convallaria*) is here taken as a type. As an alternative the study of *Erythronium* is also outlined.

Convallaria majalis, L.

A. Sketch the entire plant.

B. Does the underground portion all belong to the root system? Give reasons for conclusion. Is it highly specialized for storage of reserve material?
   1. Test a piece of it (collected in winter) for starch, Sec. 12.
   2. Cut a cross section of the most vigorous portion, examine with m.p., and decide which of the types of stem studied in Secs. 25, 26, it most resembles.
   3. In early spring note the manner in which the plant emerges from the ground.

C. In the portion above ground note first the scale leaves, and, higher up, the foliage leaves. Label these in your sketch.
   1. Are both leaf surfaces equally or unequally exposed to the light? Hold a leaf up to the light and study its venation. Describe it.

* To the Instructor: As these studies consume much time, it may be found desirable to select only a few of them. One of the longer ones, like Sec. 165 or Sec. 167, thoroughly worked out, is worth more than the whole series hurriedly done.

[1] A detailed study of the pine may be found in Sec. 138. It may, if necessary, be simplified to make it homologous with those studies of families of angiosperms which here follow by omitting some of the histological work and the larger part of the details about the process of reproduction in gymnosperms.

2. Cut a cross section of a leaf, examine with m.p., and sketch a portion extending from the one epidermis to the other. Describe in a few words the characteristics of the leaf structure as compared with any others you have studied.

D. Note the mode of origin of the peduncle (*scape*).

E. Note the position of the flowers. Uses of this. Describe the flower, making a diagram of a longitudinal section and a cross section.

F. If fruit of the preceding season (in alcohol or formalin) is at hand, describe it. Does it reproduce mainly by seed or by other means?

G. Does this appear to be a sun plant or a shade plant? Reasons for conclusion. Is it a mesophyte or a xerophyte? What is its habitat in a wild state? Look for insect visitors. What attractions has the flower for these? Could it be readily pollinated without them?

Erythronium.

A. Sketch the entire plant.

B. If possible, dig away the earth with a trowel and make a diagram to show how the aërial parts stand above ground and how the underground portion is distributed. Describe the bulbs. Of what use are they?

1. Test one for starch, Sec. 12.
2. Can they be drawn from the ground by pulling the leaves and scape? Advantage? Has the plant a stem?

C. How do the young leaves emerge from the ground? What is their position when full grown with reference to light? Strip off epidermis from both surfaces and study with m.p. Differences? Explain.

D. What is the position of the fully opened flower? Advantage? Describe the flower.

1. Make a diagram of the cross section.
2. Make a diagram of the longitudinal section.
3. Look for nectar and nectaries. What insect visitors frequent the flower?

E. Do many seeds ripen? Can the plant reproduce itself otherwise than by seed?

F. Is the species of *Erythronium* studied a sun plant or a shade plant, a mesophyte or a xerophyte? Do its leaves and blossoms mature earlier or later than those of the larger plants amid which it grows? Advantages? What becomes of the leaves during the summer?

Plants of the lily family are readily distinguished from those of the nearest related families. They differ from the rushes on the one hand in having a well-developed, not membranous, perianth, and from the members of the amaryllis family and the iris family on the other by having hypogynous flowers. The *Liliaceæ* are divided into about ten subfamilies of very

unequal numbers. Some of the most obvious differences between these relate to the bulb or rootstock or the aërial stem.

Erythronium belongs to the lily subfamily and *Convallaria* to the asparagus subfamily.

Give reasons why the lily, dogtooth violet, tulip, trillium, asparagus, lily of the valley, hyacinth, crown imperial, and onion should be classed as of the same family.

163. Family Ranunculaceæ. Study any obtainable kind of buttercup, discarding such of the following questions as do not apply to the species in hand.

Ranunculus abortivus.

A. Sketch the entire plant.

B. Describe the root system.

C. Cut the stem across and note any peculiarity of its structure.

D. Note the three kinds of leaves, — "root leaves" at the base of the stem, ordinary leaves, and involucral leaves. Sketch one of each kind. What is the advantage of having the upper leaves parted into narrow divisions? of having the root leaves long-petioled?

E. Describe the floral organs.

1. Make a diagram of a longitudinal section of the flower. How much apparent union of parts of the same or of different circles is there?
2. Do all the anthers mature together? Advantages?
3. Do the stigmas and anthers mature together? Advantage?
4. Look for the nectar and nectar glands. What insect visitors occur? Are the flowers self-pollinated?

F. Study a head of mature akenes and describe it. Does seed mature abundantly? Why cannot this species become a troublesome weed like *R. bulbosus* or *R. acris*?

G. Test the flavor of any species of *Ranunculus* that you can get, by biting the fresh stem. Explain uses. Does this buttercup seem to be a more or less highly specialized plant than the columbine (*Aquilegia*) or the larkspur (*Delphinium*) of the same family? In what respects?

164. Family Rosaceæ. Study any kind of rose except the cultivated varieties with double flowers, or any kind of cherry or plum.

Rosa humilis.

A. Describe the height and mode of branching of this rose. Are all specimens equally prickly? Where do prickles occur?

B. Sketch a typical leaf (of five leaflets). How and how much do the leaves vary? Study a cross section of a leaf with m.p. to find out how much the epidermis protects against excessive transpiration. If convenient, compare with a greenhouse species, e.g. tea rose.

C. Describe the occurrence of the flower buds. When and for how long do the flowers open? Advantage? Look for insect visitors. What do these collect? Are roses probably dependent on insect pollination? Make a diagram of a longitudinal and of a cross section of the flower.

D. Study fruit of the rose from material in alcohol or formalin. Are fresh rose hips edible? Are the seeds? How long do rose hips remain on the branches? Advantage? Have you seen birds eating them? At what season? What becomes of the seeds when birds eat the pulp of the fruit? Are most rosebushes browsed by cattle? Reasons?

E. Is this rose adapted to a moist or a dry habitat? How?

Prunus serotina, wild black cherry.

A. What is the shape of full-grown trees? Size of the largest ones in your vicinity?

B. Sketch and describe the leaves.

C. Sketch a flower cluster. Do cherry and plum trees blossom before or after the leaves develop? Are they all alike in this? Advantages of blossoming first?

1. Make a diagram of a longitudinal and a transverse section of the flower. How many ovules are there? Do all mature?
2. Look for nectar and nectaries. Do the anthers all mature together? Are there insect visitors?

D. Is the fruit edible? Do birds gather it? What evidence is there of wide distribution of the seeds? Why do cherry trees often grow beside fences?

The rose family (as found in temperate regions) is divided into four subfamilies, — *Spiræoideæ*, *Pomoideæ*, *Rosoideæ*, and *Prunoideæ*. Familiar representatives of these are the *Spiræa*, the apple, the rose, and the cherry. The most obvious differences between these subfamilies depend on the development of the receptacle and the way in which the carpels are borne on or within it. In the *Spiræoideæ* the receptacle is flattish and the carpels are borne on its surface. In the *Pomoideæ* the flowers are epigynous and the carpels appear to be grown fast to the hollow inner wall of the receptacle. In the *Rosoideæ* the carpels are in some genera (as in the rose) moderately attached to the interior of a hollow receptacle, and in other genera (as in the raspberry, the blackberry, and the strawberry) they are borne on the outside of a more or less elongated and thickened receptacle. In the *Pomoideæ* there is often but one carpel, which ripens only a single seed, inclosed in a fleshy stone fruit.

Is there any general similarity in the size, habit, and degree of woodiness of rosaceous plants?

How could all rosaceous plants be roughly classified as regards their leaves?

What can you say of the economic importance of the family? Make a list of all the cultivated *Rosaceæ* that you know and state for what each is valued. Uses of some wild species?

165. Family Leguminosæ. The black locust (*Robinia Pseudacacia*), one of the vetches (*Vicia* or *Lathyrus*), or the common pea (*Pisum*) are good types for study.

Robinia Pseudacacia.

A. Sketch a well-grown tree (best before the leaves appear).

B. Examine the roots for tubercles. In the locust, as in the *Leguminosæ* generally, these serve an important purpose in manufacturing soluble nitrogen compounds for the use of the plant.

C. Sketch a twig to show the arrangement of the thorns.
   1. What are the thorns? How related to the winter buds? Why?
   2. Are the twigs mature and alive to their tips? How is the growth of the twig continued in the spring? What other trees or shrubs do you know with the same characteristics?
   3. Cut off a large branch of locust. Is there much distinction between sapwood and heartwood? For what is the wood most valuable? What other woods are notable in the same way?

D. Sketch a locust leaf.
   1. Is the number of leaflets constant?
   2. Study and report on the positions of leaves on horizontal and vertical twigs. Explain.
   3. Study the leaflet position: (*a*) on outer branches in sunlight; (*b*) on inner branches or in dull weather; (*c*) at night.
   4. Sketch a leafy twig as seen in positions of (*a*), (*b*), and (*c*). Explain the use of each position.

      Try to ascertain by studies of the leaves on the tree what percentage of the noon illumination on a perfectly sunny day is necessary to produce position (*a*) and what to produce position (*b*). (For method of measuring relative intensities of illumination see Exp. XXVIII.)

E. Note the time of appearance of the flowers relatively to that of the leaves. Is it the same in all individuals? In southern Italy the flowers usually appear before the leaves. Advantage of this?
   1. Sketch a twig with a flower cluster in its natural position.
   2. Sketch an entire flower and another dissected. Why is a flower of this shape called papilionaceous?
   3. Make a diagram of the longitudinal section.
   4. Make a detailed drawing of the longitudinal section of a large flower bud, two to four times natural size. Show plainly the beard of hairs below the stigma.

5. Remove from a just-opened flower all the floral organs except the pistil and make an enlarged drawing of the pistil, side view. Where is pollen accumulated on it?
6. Which matures earlier, stigma or anthers?
7. Watch a bee visiting the flowers and note what happens when she alights on the wings. Why are the wings and keel fastened together? Imitate the action of the bee by pressing the wings and keel downward. Explain why cross pollination is almost sure to occur.
8. Make a list of all the attractions which this flower has for insects. What insect visitors have you observed?

F. Study the ripe fruit of the locust. What becomes of it in the autumn? Are the seeds likely to be destroyed by animals? Reasons? Part of the locust seeds of each season grow within a year, but others do not grow until succeeding years. Advantage of this?

G. Write a brief essay on the ecology of the locust, explaining all its adaptations with reference to utilizing bacterial symbionts, to light supply, to browsing animals, to pollinating insects, and to reproduction by seeds.

Lathyrus odoratus, sweet pea.

A. Sketch the entire plant. Study the distribution of the hairs on its surface. Of what use may these be? In southern Europe, where this plant is common in a wild state, snails are among the most important enemies of vegetation and they rarely attack hairy plants.

B. Examine the roots for tubercles. What kind of a climber is it? How does it climb? Why?

C. Sketch several leaves with tendrils in various stages of development. How does the tendril pull the plant toward any support on which it fastens?

D. Sketch a bit of stem with a flower cluster in its natural position. Make a detailed study of the flower as described under *Robinia*.

E. Study the ripe fruit of the sweet pea. Are the pods likely to be eaten by animals? Reasons? Can you find out how the seeds are distributed? Explain how the vetches are equipped to hold their own as dwellers in thickets and in tall grass or among other large herbaceous plants.

The family *Leguminosæ* is a very large and important one, divided into three subfamilies — *Mimosoideæ*, *Cæsalpinioideæ*, and *Papilionatæ* — whose characteristics are based on the structure of the flowers. The first, the *Acacia* subfamily, is mostly tropical; the second, the *Cassia* subfamily, contains three quite familiar North American genera, — *Cassia* or wild senna, *Cercis* or redbud, and *Gleditschia* or honey locust. Most of our familiar *Leguminosæ*, however, belong to the third subfamily, *Papilionatæ*, readily recognizable by their papilionaceous flowers. Make out a list of those which you know are useful or ornamental and give their uses.

166. Family Violaceæ. Study any species of violet; if some of the points suggested in the following outline do not fit the species in hand, omit them.

Viola palmata.

A. Sketch the entire plant.

B. Has it any stem? Explain.

C. Sketch one of the first leaves of the season and a later one.

D. Sketch a flower in profile in its natural position.

1. What kind of symmetry has it? Does the flower, seen from in front, appear open or closed? How much of the stamens and pistils can be seen?
2. Make a diagram of the cross section of the flower.
3. Remove the petals. Is the spur a part of the calyx or the corolla? It serves as a nectary.
4. Note the two nectar glands which project from stamens into the spur.
5. Make a sketch of the magnified pistil, surrounded by the stamens.
6. Are the filaments united? the anthers? Do the anthers discharge inwardly or outwardly? Is the pollen dry or sticky?
7. Note that the pollen when shed collects in a sort of cone formed by the united anthers, which is closed at the narrow end by the pistil. When a visiting insect, as a bee seeking nectar, thrusts its tongue into the small end of the cone, what would become of any pollen which the insect brought with it? Would other pollen be carried away? Result?
8. Thrust a slender, moistened toothpick gently into the opening of the corolla, and after withdrawing examine it with a lens. Result?
9. What means have violets of advertising their supply of nectar? Compare the attractiveness of several species.
10. Look for insect visitors. Do they all explore the interior of the flower, as shown in *Principles*, Fig. 325?
11. Many violets form most of their seed from apetalous cleistogamous flowers (see *Principles*, Sec. 408). Is *Viola palmata* one of these? Study specimens in late summer or early fall to determine this point. What are some advantages of cleistogamy? disadvantages? How would a plant with some insect-pollinated flowers and other cleistogamous ones avoid the disadvantages mentioned?

E. What mechanism have the capsules for distributing seeds?

F. Are any violets moderate xerophytes? Are any hydrophytes? Does the species studied belong to either class?

The *Violaceæ* constitute a small and unimportant family, but the flowers are decidedly interesting from the perfection of their adaptation for cross and self pollination.

167. Family Compositæ. Most of the genera of this family found in the United States are summer or autumn flowering. Two common genera which flower in spring are *Taraxacum* and *Erigeron*.

Taraxacum officinale, common dandelion.

A. Sketch the entire plant.

1. Notice the rosette formed by the leaves, all borne close to the ground or even pulled below the surface by contraction of the tap root. What is the use of this shortening of the tap root? What other plants show it?
2. Sketch the plant as seen from above. How much do the leaves overlap and shade each other? How much do they interfere with the growth of grass in a lawn? Advantages?
3. Taste the root and leaves. Use of this taste? Are the leaves easily injured by frost?

B. 1. Sketch the slender scape with its head of flowers. How do you know that the whole yellow "dandelion" is an inflorescence and not a flower? Advantage of grouping flowers in a head?
2. Changes in length of scape as it grows older? Advantages?
3. Describe the involucre.
4. What is its condition in the bud? in a fully opened head? in a head that is past blooming? in a head when the fruits ("seeds") are beginning to disperse?
5. Of what use is the involucre?
6. Make a longitudinal section through a newly blooming head and note whether all the flowers mature together.
7. The cushion-like expanded extremity of the scape, from which the flowers spring, is a common receptacle for all the flowers of the head.

C. 1. Note and describe the change in form of the corolla as the buds open.
2. Decide from the number of teeth at the tip of the corolla and the number of stamens what is the numerical plan of the flower.
3. Slit open the corolla of a bud with a needle and scalpel, or two needles, under the magnifying glass, and note the structure of the flower. How many stamens are there? From what part of the flower do the filaments spring? Are the anthers attached to each other? How do they open? Position of the anthers relative to the style? How many branches has the stigma? What is their form and position (*a*) in the bud? (*b*) in the newly opened flower? (*c*) in the flower just before withering? What portion of the stigmas is hairy?
4. When the stigmas emerge from among the anthers what do they bring with them? Importance of this? Could the movements of

the stigmas cause self-pollination? At what period in their development? Advantages of this?

5. Look for nectar. How high does it rise in the corolla tube? Accessibility to small insects?
6. How many kinds of insect visitors do you find?[1]
7. Is the head open on sunny and cloudy days alike? Is it open all night? Advantages? Mark a head by tying twine loosely about the scape and note how long it remains in blossom, how long it remains closed after blossoming, and how long after reopening the last fruits are dispersed.

D. Sketch a fruit somewhat magnified and label the parts. Test the traveling powers of some akenes in a gentle breeze.

E. Study the distribution of dandelion plants in your neighborhood, state where they thrive best, and give reasons.

F. Write a brief essay on the ecology of the dandelion, discussing:

(1) Relations to other plants.
(2) Relations to leaf-eating insects and grazing animals.
(3) Relations to pollinating insects.
(4) Relations to weather.
(5) Distribution of seed.

Erigeron philadelphicus, common fleabane (or other species).

A. Study the fully opened heads and make out a list of resemblances to and differences from the head of the dandelion. Note that every flower in the dandelion head has a strap-shaped corolla, and is bisexual.

1. Where are strap-shaped flowers of the fleabane? Are they bisexual?
2. Sketch under the lens a tubular flower.

B. Look for insect visitors.

C. Discuss the relative equipment of the dandelion and the fleabane for success in life.

The family *Compositæ* is the largest family of seed plants, comprising about eleven thousand species. It is usually considered to be the highest family. Not many *Compositæ* in temperate climates are shrubby or tree-like, but as herbs they show the greatest diversity of form and ecological characteristics. As a rule, they are extremely successful in maturing and distributing seed, and for this and other reasons constitute very formidable weeds.

Make a list of some of the commonest weeds of this family in your neighborhood.

References. *Principles*, Chapters XXXII, XXXIII; Strasburger, Noll, Schenck, Karsten, 1; Warming-Möbius, 37; Engler, 36; Knuth-Davis, 62; Kerner-Oliver, 2.

[1] Nearly a hundred species have been noted in a single locality.

BOTANICAL MICROTECHNIQUE

168. Introduction. This section will describe the technique of a number of well-known histological and cytological methods involving the preparation of material. Its object is to present simple and clear descriptions of tried methods that can be depended upon to give good results. Detailed treatments may be found in a number of treatises.[1] The best general accounts of histological methods in English are those in Strasburger-Hillhouse, 6, which is based on Strasburger's *Das botanische Praktikum*. The subject-matter of the following brief account will be taken up under the following headings:

General reagents employed in temporary preparations.
Some special reagents for microchemical and other tests and temporary preparations.
Killing and fixing.
The preservation of material.
General staining methods.
Mounting in balsam and glycerin.
Imbedding in paraffin.
Sectioning.
Staining on the slide.

GENERAL REAGENTS EMPLOYED IN TEMPORARY PREPARATIONS

169. General reagents employed in temporary preparations.

A. *Iodine solutions.* Dissolve 5 grams potassium iodide in 100 cc. distilled water, and add 1 gram of iodine. This gives a good strength for most purposes. It may be diluted if desired, and should be used of half this strength, or weaker, when the color of the solution might interfere with the clearness of vision, as when zoöspores are stained to show their cilia.

Iodine solutions kill protoplasm quickly, staining it a deep brown, especially the nucleus and chromatophores. They furnish the simplest

[1] Zimmermann-Humphrey, *Botanical Microtechnique*, Henry Holt & Co., New York, 1893. Poulsen-Trelease, *Botanical Micro-Chemistry*, S. E. Cassino & Co., Boston, 1884. Chamberlain, *Methods in Plant Histology*, The University of Chicago Press, Chicago, 1905.

tests for starch (Sec. 12), coloring the grains blue, or a deep brown if the solution be too strong. Cellulose (Sec. 12) is generally stained yellow or brown, which changes to blue if a strong solution of sulphuric acid be applied after the iodine.

B. *Chlorzinc iodine.* This is a troublesome reagent to prepare, but the best test for cellulose. Dissolve zinc in pure hydrochloric acid and evaporate the solution (with metallic zinc present in it during the process) to the density of sulphuric acid. Add as much potassium iodide as the solution will dissolve, and finally as much metallic iodine as it will take up. The solution will keep better away from the light. Chlorzinc iodine stains pure cellulose a clear blue or violet. It reacts best on preparations in water.

C. *Potash solution.* A 5% solution of potassium hydrate, or caustic potash, in water is an excellent clearing and softening agent. A 15% solution is necessary for some subjects, as firm leaf sections. The potash solution may be neutralized by washing the sections in commercial acetic acid and then mounting them in the latter.

Potash solutions must be kept tightly stoppered. Rubber stoppers answer well; glass ones should be covered with paraffin, otherwise they are likely shortly to become stuck beyond the power of removal.

D. *Acetic acid.* A 1% solution of glacial acetic acid in water will fix and frequently bring out clearly the nucleus and other protoplasmic structures of a cell. Beautiful temporary preparations may then be made by staining with gentian violet (see F) or methyl green (Sec. 186, B).

E. *Eosin.* A strong solution of eosin in water is the most useful. Alcoholic solutions may be employed when the preparation is in alcohol. This stain has the peculiar advantage of coloring protoplasm alone, leaving the cell wall unaffected.

F. *Gentian violet.* A deep violet solution in 1% acetic acid is a good strength for temporary staining of fresh material, or after fixing with 1% acetic acid (see D).

G. *Alcohol.* Alcohol has its chief value for temporary preparations in driving out air bubbles from material which will not wet easily in water, as, for example, the mycelium of fungi.

H. *Distilled water.* Temporary preparations of living plants are mounted in tap water, or that in which they live, if aquatic. Distilled water is used when the preparations are made from preserved material.

I. *Glycerin.* A solution one third glycerin and two thirds distilled water is very useful in preserving temporary preparations. A drop or two placed at the edge of the cover glass will prevent the preparation from drying up. This solution, or one considerably stronger, is also used for permanent preparations when inclosed in a cement ring (Sec. 188).

SOME SPECIAL REAGENTS FOR MICROCHEMICAL TESTS AND TEMPORARY PREPARATIONS

170. Some special reagents for microchemical tests and temporary preparations.

A. *Alkanet root tincture.* Add enough bits of alkanet root to 95% alcohol to color it deep red. The solution serves as a test for oils and resins (Sec. 12), coloring them red.

B. *Ammonia.* Ordinary commercial ammonia water is used after treatment of sections, etc., with nitric acid to give the xanthoproteic reaction (Sec. 12), coloring proteids yellow or orange.

C. *Chloral hydrate.* A solution of eight parts of chloral hydrate in five parts of water by weight forms an excellent clearing reagent for growing points and pollen grains.

D. *Chloroform.* Removes oil from sections of seeds which are to be examined for aleurone grains.

E. *Fehling's solution.* This reagent may be bought of dealers in chemicals. It is usually made up in the form of two or three solutions, which are to be mixed only at the time of using. The following formula is convenient, and keeps well in a cool place. Dissolve 34.64 grams pure crystallized copper sulphate in 200 cc. of distilled water. Mix the solution with 150 grams of neutral potassium tartrate dissolved in about 500 cc. of a ten per cent solution of sodium hydrate. The whole is then to be diluted with water to 1 liter, and 100 cc. of glycerin added. The solution serves as a test for sugar (Sec. 12).

F. *Millon's reagent.* Dissolve metallic mercury in its own weight of c.p. concentrated nitric acid and dilute the solution with its own volume of distilled water. This reagent swells cell walls and usually colors proteids (Sec. 12) a characteristic brick red.

G. *Nitric acid.* C.p. nitric acid, slightly or not at all diluted, is used as a test for proteids (Sec. 12). It is also used in Schultze's macerating mixture (see M).

H. *Olive oil.* This is used as a mounting fluid for sections of seeds with aleurone grains.

I. *Phloroglucin.* 1–5% solutions in water or alcohol are used as a test for lignified tissue (Sec. 12).

J. *Potassium chlorate.* This is used as an ingredient of Schultze's macerating mixture (see M).

K. *Potassium permanganate.* A 4% solution of this compound in water is used as a stain to distinguish roots from stems in very young seedlings.

L. *Safranin.* A saturated or sometimes a half-saturated aqueous solution of this stain is valuable for differentiating tissue elements, e.g. in stem

sections or leaf sections. There are several good formulæ for safranin stains (Sec. 184).

M. *Schultze's macerating mixture.* This mixture is used to disintegrate tissues (e.g. wood) to obtain individual cells. The material to be treated should be in small bits cut lengthwise. Place the sections in a test tube and pour on just enough strong nitric acid to cover the material. Add a few crystals of potassium chlorate and heat gently until bubbles are given off and the substance treated becomes white. If violent action occurs and abundant reddish fumes are evolved, repeat the operation with fresh bits of the substance to be macerated, using less chlorate. The process must be conducted out of doors or under a hood, as the acid vapors produced are very corrosive and injure microscopes and most metallic apparatus. When the maceration is finished the fibrous material left should be thoroughly rinsed with successive portions of water until all traces of acid are removed. It may then be teased apart with needles and preserved in glycerin, and portions mounted for examination as required.

N. *Sugar.* Cane sugar is used in the preparation of solutions for the culture of pollen tubes (Experiment XLII), algæ (Sec. 200), and germinating spores of mosses and ferns (App. 19 and App. 20).

Solutions of the required strengths can be made by weighing out the necessary amounts of granulated sugar and adding to measured or weighed amounts of tap water. One and a half per cent of gelatin may, with advantage, be added to most of the solutions for the culture of pollen tubes.

KILLING AND FIXING

171. The principles of killing and fixing. Fixing is the preservation of the structure of protoplasm immediately after death as nearly as possible like that of the living cell. Killing and fixing are generally accomplished by the same fluid. Fixing agents have in their composition elements (as chromium, osmium, platinum, etc.) which living protoplasm normally never or but rarely encounters, or severe combinations of poisons that are utterly foreign to it. The ability of a killing fluid to fix undoubtedly rests on its power to subject protoplasm to a shock so sudden and great that there is little or no time for great structural changes to take place, while the reagent itself must not cause disorganization. Fixed material must later be preserved, a process involving quite different methods and reagents (Secs. 177, 178).

172. Chrom-acetic acid. The combination of chromic and acetic acids in various proportions has proved to be a very satisfactory general fixing agent, and is among the cheapest. Three grades of chrom-acetic acid will be found

useful, — a *weak*, a *medium*, and a *strong*. Any shrinkage of the cell contents during fixation indicates that the solution is too strong in chromic acid, which has a tendency to contract the protoplast, partially compensated by the acetic acid which is employed because of its tendency to swell the cell contents.

A. *Weak chrom-acetic acid*[1]:

1% chromic acid	25 cc. making .25% chromic acid.
1% acetic acid	10 cc. making .1% acetic acid.
distilled water	65 cc.
	100 cc.

This is generally the most satisfactory strength of chrom-acetic acid for algæ and fungi, and the more delicate structures of the liverworts and mosses will be excellently fixed by it, likewise fern prothallia.

B. *Medium chrom-acetic acid:*

1% chromic acid	70 cc. making approximately .7% chromic acid.
glacial acetic acid	.5 cc. making approximately .5% acetic acid.
distilled water	30 cc.
	100.5 cc.

A good fluid for most work on the histology of the pteridophytes and seed plants and the firmer structures of mosses and liverworts.

C. *Strong chrom-acetic acid:*

1% chromic acid	100 cc. making approximately 1% chromic acid.
glacial acetic acid	1 cc. making approximately 1% acetic acid.
	101 cc.

This solution may prove more satisfactory than medium chrom-acetic acid when the tissue is dense or with very heavy cell walls.

The determinations of the proper relative strengths of chromic and acetic acids become matters of experience and experiment which must be tested with untried subjects, but those who use this fixing fluid are soon able to judge very accurately the strength and time necessary for good results. Chrom-acetic acid keeps perfectly, and costs so little that it may be made up in large quantities; it is the most useful general fixing agent in the laboratory. It should be employed in liberal quantities, perhaps one hundred times the bulk of the material, or in such amounts that the fluid is not noticeably discolored by the material.

[1] The formulæ in this account are generally given in terms of 100 cc. The proportions may be multiplied by tens and hundreds for larger quantities. Chrom-acetic acid is so useful a reagent that a laboratory should always have a stock supply. Another plan is to keep on the same shelf or table a large bottle of 1% chromic acid, another of 1% acetic acid, and a small bottle of glacial acetic acid, together with a large and small graduate and the formulæ posted on the wall. Solutions may then be made up at any moment.

Material is generally left in chrom-acetic acid for twelve hours or more, but very delicate structures require only an hour or two. Some algæ with soft cell walls, such as *Polysiphonia*, will go all to pieces if left in the weak formula more than five or ten minutes. Solutions employed upon the marine algæ must be made up in salt water instead of distilled water, and the fixed material must also be washed in salt water. Tissues with hard or very firm cell walls are improved by being left for longer periods, perhaps several days, in the fluid, for the chromic acid acts on the cell walls, softening them somewhat.

Material fixed in chrom-acetic acid must be washed thoroughly before being carried up into alcohol for final preservation (Sec. 178). This may be done most satisfactorily with firm tissues in a gentle stream of tap water circulating through the vessel (a wide-mouthed bottle with a piece of gauze tied over the mouth to hold the material within is convenient). Washing may occupy several hours or, with firm tissues, a much longer time without danger. It is necessary to get all of the chromic acid out of the material, otherwise a precipitate will be formed by the alcohol and the protoplasm will not stain well.

It is not generally known that the chromic acid can be washed out by running the material through the grades of alcohol to 70% (Sec. 178), provided the bottle of material is *kept in the dark*. The precipitate referred to in the paragraph above is only formed by alcohol in the presence of light. The 70% alcohol must of course be changed until there is no trace of chromic acid. This method works especially well with small objects and saves much time and the somewhat difficult operation of washing small objects in water.

173. Chrom-osmo-acetic acid (Flemming's fluid). The most successful of the formulæ containing chromic, osmic, and acetic acids were developed and perfected by Flemming and are accordingly called Flemming's fluids. The addition of osmic acid to the chrom-acetic basis gives somewhat better fixation of material than chrom-acetic acid alone. This better fixation appears in the cytological details of nuclear division and in a more brilliant reaction of material to the stains safranin and gentian violet, which with orange G form a group often used together as a triple stain (Sec. 199, D) after this fixing agent. Flemming's fluids penetrate slowly, and material should be cut up into small pieces or slices, perhaps an eighth of an inch in thickness, to obtain the best results. The expense of the osmic acid rather precludes the use of these fluids for general morphological and histological studies where fortunately the cheap chrom-acetic formulæ are in the main quite satisfactory. Flemming's fluids do not keep in the light, and it is best to make them up fresh just before fixation. Solutions of osmic acid must be kept in the dark.

A. *Weak chrom-osmo-acetic acid (Weak Flemming):*

1% chromic acid	25 cc. making .25% chromic acid.
1% acetic acid	10 cc. making .1% acetic acid.
1% osmic acid	10 cc. making .1% osmic acid.
distilled water	55 cc.
	100 cc.

This well-known formula is used for the algæ and fungi and delicate tissues of the higher plants. It has the same strength as weak chrom-acetic acid but with osmic acid added. Half the amount of osmic acid in the above formula gives, according to our experience, better results with many algæ and fungi.

B. *Strong chrom-osmo-acetic acid (Strong Flemming):*

1% chromic acid	75 cc. making .75% chromic acid.
glacial acetic acid	5 cc. making 5% acetic acid.
2% osmic acid	20 cc. making .4% osmic acid.
	100 cc.

This formula has a medium strength of chromic acid but an exceptional strength of acetic and osmic acids. It may easily be modified by varying the amounts of its components. Thus Mottier recommends for anthers the following proportions: 1% chromic acid, 80 cc.; glacial acetic acid, 5 cc.; 2% osmic acid, 15 cc. Strong Flemming naturally finds its use on the same sort of subjects as require the medium or strong formulæ of chrom-acetic acid, as for example the firmer tissues of the higher plants.

Material fixed by Flemming's fluids must be washed to remove the chrom-acetic acid, as described in the previous section. The osmic acid always blackens the material, but this discoloration is not treated until just before staining (Sec. 198), when the preparations are bleached with hydrogen peroxide. The chromic acid must be dissolved in sea water, when these fluids are used upon marine algæ, and the material also washed in sea water.

174. Absolute alcohol. The fixing fluids based on chromic acid penetrate rather slowly, and consequently very dense tissues or structures with heavy hard cell walls are sometimes not at all well fixed by them, the protoplasts appearing shrunken. There is also occasional difficulty in immersing or wetting material in these water solutions. For such material some of the fixing fluids based on alcohol are preferable. The best of these are absolute alcohol and Carnoy's fluid.

Absolute alcohol alone is not an especially good fixing agent except for very small objects, which it can penetrate almost instantly. Material is, of course, ready very quickly for preservation in 85% alcohol, or for the process of imbedding in paraffin.

175. Carnoy's fluid.

absolute alcohol	60 cc.
chloroform	30 cc.
glacial acetic acid	10 cc.
	100 cc.

This is a strong fixing fluid which penetrates very rapidly, and consequently should only be used for a few minutes, — ten to thirty minutes is probably long enough for most subjects. There is always danger of leaving material too long in it. The material is washed in changes of absolute alcohol until there is no odor of acetic acid, and is then best imbedded at once, but may be transferred to 85% alcohol for preservation. The staining of chromosomes after Carnoy's fluid is sometimes very brilliant, but spindle fibers and other kinoplasmic structures are apparently less perfectly preserved than by the chrom-osmo-acetic formulæ.

If the subject be very resistant to penetration, as for example the megaspores of the pteridophytes, the proportionate amount of acetic acid may be greatly increased. Thus two parts glacial acetic acid, one part absolute alcohol, and one part chloroform have been recommended as giving good results for the spores of *Selaginella*. However, even in these cases, long treatment with medium or strong chrom-acetic acid, especially if applied hot, aided by mechanical cutting or pricking of material, to assist penetration, will frequently give better results than Carnoy's fluid.

176. Concluding suggestions on fixing. It is important to facilitate mechanically, in every way possible, the rapid penetration of the fixing fluid. Thus an ovary of a lily should be pared along the angles and then sliced in pieces three eighths of an inch thick or cut lengthwise. Small objects, such as filamentous algæ, may be examined at various stages in the process of fixation to see if the cell contents are in good condition. Material that must be sectioned cannot, however, be so easily observed, and shrinkage may occur, which was not caused in the fixing, but at some later stage in the manipulation leading to sectioning or staining. Consider results critically, and when unsatisfactory attack the problem as one of physics and chemistry, and find just where the methods failed. Close attention to these details will soon give a sure command of a few simple methods of fixing which are likely to give satisfaction.

THE PRESERVATION OF MATERIAL

177. Alcohol. Material collected for general morphological study may be placed at once in 95% alcohol. It must later be transferred to a lower grade, such as 70%, or it will become very brittle. Alcohol mixed with glycerin, half and half, or one fourth glycerin, will keep material from becoming

brittle, and is especially good for firm structures that are to be sectioned free-hand, such as the various parts of seed plants.

Alcohol is the best all-round preservative. Other fluids have appeared from time to time as rivals, as for example formalin, but they none of them have supplanted it. It is somewhat uncertain whether denatured alcohol, now on the market, will be just as good as the pure alcohol for preservative purposes, and it should be used with some care until its effects are known.

178. Bringing fixed material into alcohol. Botanists are coming to depend more and more upon fixed material even for general morphological studies, since it is very little trouble and expense to fix in chrom-acetic acid, and the superior results are worth the attention required. This is especially true of type material of the thallophytes and bryophytes.

Material fixed in chrom-acetic acid or in chrom-osmo-acetic acid (Flemming's fluids) must be washed as described in Sec. 172, and then passed, or "run up," through several grades of alcohol to 70% (or 85% if the material is delicate), where it may rest indefinitely. It is well to begin with 15% alcohol and pass successively through 25%, 35%, 50%, to 70%. Small objects such as lily anthers will not require more than an hour in the lower grades. They should remain, however, twice as long in the 35% and 50%. Larger objects must remain from four to eight hours in each grade. The process should be planned so that material is not left for so long a time as over night in a grade of alcohol below 50%, and it should not remain in 50% longer than over night. Generally the entire process can be finished in one day. The grades of alcohol are made from 95%, which for general purposes is regarded as being pure.

Material fixed in fluids based on alcohol, as for example Carnoy's fluid, should be passed directly into a grade of alcohol corresponding to that in the fixing fluid.

179. Formalin. Much was expected of formalin when it appeared a number of years ago. The most important claims have not been fulfilled. It will not preserve the green color of plants in the light, and shades of red, blue, and brown are generally modified after a few months. Unless the tissue is firm it is apt sooner or later to soften or macerate ; this is especially true of the lower plants. Finally, formalin is intensely disagreeable to work with on account of its effect on the nose and eyes.

Formalin, which is about 40% formaldehyde, is added to water to make a 2-5% solution. It is convenient to carry, since a small quantity will make many quarts of the preserving fluid. If material is to be used within a short time, formalin will prove satisfactory. Material may also be transferred from formalin to alcohol, being carried up through the grades. However, its advantages are rather doubtful when chrom-acetic acid and alcohol are at hand.

GENERAL STAINING METHODS

180. Methods of staining. There are two principal methods of staining, — (1) in bulk or loose sections, and (2) on the slide. The second method generally follows the process of sectioning in paraffin, and is given special consideration in Secs. 197–199. Staining in bulk is, on the whole, much less precise in its results than the staining of microtome sections. The methods of staining in bulk also apply to sections cut free-hand (Sec. 194), either from preserved or living material.

181. Eosin. Saturated solutions in water or alcohol are used. Material is stained almost at once, and should then be transferred to 1% acetic acid for a minute (which renders the stain less soluble), after which the acid should be thoroughly washed out. Permanent preparations are generally made in glycerin after the method outlined in Sec. 188. If the preparations are to be mounted in balsam (Sec. 187), the staining should be with alcoholic solutions, or, better still, with a solution in absolute alcohol from which the material may pass directly into xylol, and there is no need of treatment with acetic acid.

Eosin does not stain cell walls and never overstains protoplasm. It is especially useful for the fungi, which are generally mounted in glycerin, and is one of the best of the quick, simple stains.

182. Iron-alum hæmatoxylin. This method, developed by Heidenhain, gives the most satisfactory results of all the hæmatoxylin stains in the differentiation of protoplasmic structure. Delafield's hæmatoxylin (Sec. 183) is a somewhat better stain for tissues, because it colors cell walls sharply. Iron-alum hæmatoxylin does not color cell walls heavily, and is consequently a very useful general stain for the algæ and fungi which are to be stained in bulk and mounted without sectioning.

Two separate solutions are used:

(1) A 2% aqueous solution of ammonia sulphate of iron (iron alum).

(2) A ½% solution of hæmatoxylin dissolved in hot distilled water.

The solution of iron alum acting as a mordant prepares the tissue to take up the hæmatoxylin. Bring the material from water (running it down through the grades of alcohol, if preserved in the latter) into the iron-alum solution, which for delicate structures may be diluted to 1%. Leave in the iron alum from one to three hours, rinse for a few minutes in water, and place in the hæmatoxylin solution. If the hæmatoxylin becomes too muddy, replace it with fresh. Leave the material in the hæmatoxylin from three to ten hours (over night does no harm) and then place in iron alum again. The black stain extracts rapidly, and the material must be examined from time to time under the microscope. When the stain has been extracted to the proper point, place the material in considerable tap water for a half hour, or

as much longer as convenient, to remove all trace of the iron alum. The material is now ready to be mounted in balsam (Sec. 187) or glycerin (Sec. 188). If mounted in balsam it must be carried through xylol (never oil of cloves, which fades hæmatoxylin).

The hæmatoxylin can be extracted to a point where the nucleus is practically the only structure stained, and is consequently one of the best of the nuclear stains. Such material may be counterstained (that is, stained in addition) with safranin (Sec. 184), thus differentiating the nucleus (gray or black) from the rest of the protoplasm (red). Iron-alum hæmatoxylin is probably on the whole the most satisfactory of all the staining methods for protoplasmic structures. It is subject to great latitude in the time limits, which may be set for the different stages of the process except that of extraction, which must of course be watched carefully; but these are soon learned with experience. It is perhaps the least uncertain of the stains, and although the process is somewhat long it can always be depended upon to give good results.

183. Delafield's hæmatoxylin. This stain reacts very differently from iron-alum hæmatoxylin. It stains cell walls sharply, but does not differentiate protoplasmic structures as well as the latter. It is one of the best stains for tissues of higher plants, and may be combined very effectively with safranin, as described below and in Sec. 185.

Delafield's hæmatoxylin is made as follows: a solution of 1 gram hæmatoxylin in 6 cc. absolute alcohol is added drop by drop to 100 cc. of a saturated solution of ammonia alum. Filter after exposing for a week to the air and light. Then add 25 cc. of glycerin and 25 cc. of methyl alcohol. Allow the mixture to stand for several hours (4–7), until the color is dark, and then filter. The solution should then remain two months in a tightly stoppered bottle to "ripen." The prepared stain may be purchased from dealers (Sec. 218).

Material is transferred to Delafield's hæmatoxylin from water or 25% alcohol. Staining will take place rather rapidly, requiring from a few minutes to an hour or more. The stain may be diluted to half or a fourth of the above strength, and the staining, although longer, is frequently better. Wash the material in tap water until a rich purple color develops. If the sections or other subjects are overstained, or if a precipitate is formed when the material is placed in alcohol, rinse in acid alcohol ($\frac{1}{10}$ cc. hydrochloric acid in 100 cc. 70% alcohol). The acid alcohol takes out the color, which may thus be extracted until the nucleus alone remains stained. When washed in acid alcohol the material must be placed in tap water until the purple color returns. Then run up in the grades of alcohol through 95% and absolute alcohol, clear in xylol, and mount in balsam, as described in Sec. 187, or pass from water into glycerin, as outlined in Sec. 188.

Material stained in Delafield's hæmatoxylin may be counterstained with alcoholic safranin, but very good results may be obtained with the tissues of higher plants by staining first with safranin, as described in Sec. 185.

184. Safranin. There are various kinds of safranin sold, some of which dissolve more readily in water and some in alcohol. The stain should always be placed in its appropriate solvent. A 1% solution in water is a good strength, and a saturated solution in 95% alcohol mixed with an equal volume of water, making a 50% alcoholic solution, is also good. The alcohol solutions are the most convenient. Anilin safranin is prepared from a saturated solution in 95% alcohol mixed with an equal amount of anilin water (made by shaking anilin oil in distilled water, when a small percentage of the oil is taken up by the water). Anilin safranin is considered by some to be the best of the safranin stains.

Safranin colors cell walls as well as protoplasm. It is therefore a general stain, but when properly extracted it may be made to differentiate certain nuclear structures sharply (chromosomes and nucleolus), and is much used in staining on the slide, especially in combination with gentian violet and orange G (Sec. 199, D). Material may remain in safranin from one hour or less to twelve hours or more. The stain is extracted in 50% alcohol until the desired coloration is obtained, or, if very much overstained, the material may be placed in acid alcohol ($\frac{1}{10}$ cc. hydrochloric acid in 100 cc. 70% alcohol). The acid alcohol, if used, must be thoroughly washed out.

185. Safranin and Delafield's hæmatoxylin. Safranin followed by Delafield's hæmatoxylin is an excellent stain for the tissues of higher plants, whether in free-hand or microtome sections. Sections cut free-hand from fresh material may be fixed for 10–15 minutes in absolute alcohol or medium chrom-acetic acid (Sec. 194); those from preserved material may be stained at once. They should remain in the safranin several hours (over night). Wash in 50% alcohol (acid alcohol if desired) until the stain is extracted from all parts except lignified cell walls, as in fibro-vascular bundles. Remove the acid alcohol if used. Stain in Delafield's hæmatoxylin for several minutes (1–30). Wash in tap water, or extract the stain if necessary in acid alcohol (as described in Sec. 183), which must be followed by tap water until the stain is purple. Carry through 95% alcohol, then absolute alcohol, clear in xylol, and mount in balsam. Sections cut on the microtome are stained on the slide (Sec. 197) in the same manner as described above.

186. Other anilin stains. There are numerous anilin dyes of great value in special cases, but few of them have such general usefulness as eosin, safranin, and gentian violet. The following, however, are important.

A. *Acid fuchsin.* A 1% solution may be made in water or in 70% alcohol. The stain acts rapidly and is very brilliant. It may be extracted in 95% alcohol from overstained material or sections.

B. *Methyl green.* A saturated solution in 1% acetic acid keeps well, or it may be made up simply in distilled water. Dilute if desired. This is a good stain for living cells, but it is especially valuable in combination with acid fuchsin, forming an effective double stain for the tissues of higher plants. Sections from preserved material may be stained at once, those from fresh material must be fixed in absolute alcohol or chrom-acetic acid (Sec. 194). Stain first with methyl green for two hours or more and wash in distilled water until the green remains in the lignified cell walls alone. Then stain with acid fuchsin for a few minutes, — not long enough to affect the lignified tissues, — and pass through 95% alcohol to absolute alcohol and into clove oil and balsam (Sec. 187).

C. *Erythrosin.* This stain is similar to eosin and may be used in saturated solutions in water or 70% alcohol. It is a good counterstain following hæmatoxylin or green and blue anilin dyes.

MOUNTING IN BALSAM AND GLYCERIN

187. Mounting in balsam. Canada balsam is the most satisfactory medium for permanent preparations. It should be used whenever possible, but there are some subjects, such as delicate filamentous algæ and fungi, which cannot easily be carried into balsam without shrinkage, or which cannot be teased apart when brought into that medium because the clearing agents such as xylol or clove oil render the filaments much less flexible. For such subjects glycerin, glycerin jelly, or Venetian turpentine are better media.

Material is carried into balsam from absolute alcohol through a clearing agent. It must first be brought up through the grades of alcohol to 95% (Sec. 178), where it is best left several hours (it may remain in 95% alcohol indefinitely). Material is then placed in absolute alcohol to remove all trace of water (dehydration). Dehydration takes from thirty minutes to an hour or more, according to the size of the object, and it is well to change the alcohol once or twice if there is much material. From absolute alcohol the material is carried into a clearing agent, clove oil or xylol being the simplest. Clove oil removes the absolute alcohol rapidly and is the better clearing agent following anilin dyes, but should *never be used after hæmatoxylin*, for its acid quality fades that stain. Xylol acts more slowly and does not affect hæmatoxylin stains. Microtome sections on the slide are handled much more rapidly through the alcohols and clearing agents, as is described in Sec. 199.

With delicate material xylol is always the safest clearing agent, because it mixes more slowly and less violently with absolute alcohol. The danger of shrinkage is lessened greatly by preparing three mixtures of absolute alcohol

and xylol: (1) one fourth xylol and three fourths absolute alcohol; (2) half and half xylol and absolute alcohol; (3) three fourths xylol and one fourth absolute alcohol. Even the most delicate material of algæ and fungi can generally be carried without shrinkage into pure xylol if passed through these grades, being left an hour or more in each. Once in xylol, material is safe from shrinkage, and it may be left in this reagent indefinitely. It is absolutely essential for good results that the dehydration be perfect. Small objects should be examined throughout the process to determine the exact time of any cell shrinkage, which may be corrected with greater care.

After being in clove oil a short time (from five to fifteen minutes), or in xylol a much longer time (several hours), the material is transferred to Canada balsam on the slide. The balsam should be so diluted with xylol that it drops readily from a glass rod. A cover glass is then gently lowered over the object with the point of a needle. The balsam will gradually harden as the xylol dries out. Air bubbles need give no concern; they will work out to the edge of the cover glass as the balsam hardens. When balsam thickens in its bottle xylol should be added; the cloudiness which may develop will soon pass away.

188. Mounting in glycerin. Material is transferred from water to a considerable quantity of a 10% aqueous solution of glycerin in a watch glass. This should not cause shrinkage. The watch glass is then protected from dust and the water allowed to evaporate until the solution is about as thick as pure glycerin. The material is now ready to be mounted and will be so soft that it can be easily teased apart. A small drop of the solution is placed on the slide, the material arranged in it, and a clean cover glass with one edge resting on the slide is carefully lowered with a needle until the glycerin runs out to the edge on all sides. This must be done so carefully that no bubbles of air are inclosed.

Practice will determine the amount of glycerin necessary to fill the space under the cover glass, which should not be more than five eighths the width of the slide. The less glycerin the better. The glycerin should not run out beyond the edge of the cover glass, although a small amount may be wiped away with a cloth moistened in alcohol. On no account must the glycerin be allowed to run over the edge of the cover glass; such a preparation is worthless. The slide is now ready to be sealed, or it may be laid away to allow the glycerin to become somewhat more dense.

The best cement is gold size. This should not be so thick that it cannot be easily spread with a brush. If too thick, thin with oil of turpentine. The gold size is generally applied at the edge of the cover glass while the slide is whirling on a turntable. A thin ring should be laid three eighths of an inch wide, half on the slide and half over the edge of the cover glass, thus sealing the glycerin within a chamber. The sealing will not be perfect unless

the cover glass and slide are absolutely dry, that is, free from any glycerin. The first ring should be thin and allowed to dry thoroughly before the second ring is applied. More may be added if necessary. Properly sealed preparations will last indefinitely, but the sealing is a delicate operation and requires some experience.

Glycerin jelly is for some subjects as good a mounting medium as glycerin, and the preparations are more durable. Transfer material from a rather thick solution of glycerin to a drop of melted jelly on a warm slide, arrange with needles and carefully lower a warm cover glass over the mount, taking care not to inclose air bubbles. It is necessary to work quickly. The cover glass may be sealed with a ring of gold size and thus strengthened.

189. Venetian turpentine. The difficulty of properly sealing glycerin preparations, together with their fragile nature, is the chief objection to the glycerin mount. A method of mounting in Venetian turpentine has recently been perfected by Chamberlain.[1] By this process material may be brought without danger of shrinkage into a medium (Venetian turpentine) which hardens like balsam and requires no sealing. The technique is somewhat long and the staining methods special, but the results are striking. The staining, so far as we have seen preparations, does not bring out the finest details of protoplasmic structure as well as such stains as iron-alum hæmatoxylin, safranin, and gentian violet. For details of this method the reader is referred to Chamberlain.

IMBEDDING IN PARAFFIN

190. The paraffin method of sectioning. There are several methods of sectioning plant tissue, all of which have their limitations, because plant structures range from those of great delicacy, as among the thallophytes and bryophytes, to the firm and hard tissues of the sporophyte generation of the pteridophytes and spermatophytes. Very firm or hard tissue cannot be cut in paraffin, and sections may be made free-hand (Sec. 194) from fresh or preserved material, but are better cut in celloidin (Sec. 195). Softer structures, such as anthers, ovule cases, and many developing organs of the seed plants, together with the gametophyte generations of the pteridophytes and almost all structures in the bryophytes and thallophytes, are sectioned most effectively by the paraffin method. Its advantages are that sections can be cut very thin, that they can easily be arranged serially on the slide, and that they can be stained with greater precision. Sectioning in paraffin is preceded by the process of *imbedding*, which involves the preliminary processes of *dehydration* and *clearing*, and *infiltration*.

[1] *Methods of Plant Histology*, p. 79, 1905.

191. Dehydration and clearing. The material to be cut is passed carefully through the grades of alcohol to 95% (Sec. 178). It should remain in 95% alcohol for at least several hours or more, and is then placed in absolute alcohol, generally in a vial, and this should be poured off and renewed after an hour or two. The material should be left in absolute alcohol from four to eight hours or over night, unless the object be very small. It ought then to be free from water (dehydrated) and ready for the clearing agent, which will remove the absolute alcohol and also dissolve the paraffin, so that the latter may replace the former throughout the tissue. The clearing agents most frequently used are chloroform and xylol. Chloroform acts more rapidly, but there is less danger of shrinkage with xylol. However, the chief danger of shrinkage lies in imperfect dehydration.

Three mixtures of the clearing agent (chloroform or xylol) with absolute alcohol are necessary to insure the gradual replacement of the latter by the former. These are (1) one fourth clearing agent, three fourths absolute alcohol; (2) half and half clearing agent and absolute alcohol; (3) three fourths clearing agent and one fourth absolute alcohol. The material is passed through these mixtures in the above order and then into the pure clearing fluid, either chloroform or xylol. When chloroform is used the material need not be left more than from four to eight hours in each mixture, and less if the object be small. If xylol is used, the material should be left at least twelve hours in each mixture, and a longer time will do no harm. It should not remain in pure chloroform more than twelve hours before paraffin is added, and a shorter time is generally better; but it may be left in pure xylol a longer time, and even a day or more with advantage. Besides removing the absolute alcohol the clearing agent renders the tissues more transparent, that is, "clears" them.

192. Infiltration. Small pieces of paraffin are now added to the chloroform or xylol to the point of saturation and beyond. At this time the vials may be placed on the top of the oven, where they will be warmed, thus allowing more paraffin to dissolve.

The best form of paraffin bath is a square or rectangular hot-water oven, with a door at the side and one or more shelves within. This should be heated by gas or by an electric coil with a thermostat arrangement to keep the oven at a constant temperature of about 52° C. The temperature may run as high as 56°, or probably higher if the dehydration has been perfect, but in general the temperature should be kept low.

Material in chloroform and paraffin is placed in the bath and the vial uncorked. The chloroform will be driven off after a number of hours, leaving the material in pure melted paraffin. This process should not be hastened; a day or two in the paraffin bath will generally give the most satisfactory results. Tasting the paraffin is the best test of the removal of the chloroform; should

it be at all sweet there is chloroform still present. The chloroform must be entirely driven off before imbedding, otherwise the paraffin will not cut well.

Material in xylol and paraffin must be treated differently from that in chloroform. Xylol cannot be removed easily by heat. Consequently the material must be transferred through solutions with less xylol in them until it is carried into pure paraffin. The simplest way is to pour off solutions and add melted paraffin, keeping the vials in the bath. The mixtures of paraffin and xylol may be saved and used again or simply left in the bath to gradually purify as the xylol is driven off. Finally the material is placed in two or three changes of pure paraffin to remove the last trace of xylol. It will do the material no harm to remain several days in the mixtures of paraffin and xylol, and structures with thick walls or coats (such as the megaspores of *Selaginella*) must be left sometimes for weeks before infiltration is completed.

193. Imbedding. The material is now in pure melted paraffin and ready to be cast in a cake. Most subjects can be cut in paraffin, which melts at a relatively low temperature, 50°–52° C. Others require a hard paraffin with a melting point of 56° or higher. In general it is better to imbed in a medium paraffin and plan to cut in a room at a cool temperature.

Petri dishes are good receptacles for the casting, or paper trays may be used. Two L-shaped pieces of metal on a glass plate are convenient, since the size of the mold may be readily adjusted to the object. The interior of the receptacle should be smeared with glycerin to prevent the paraffin from sticking. The melted paraffin is poured into the receptacle with the material and the latter is then arranged with a heated needle. Finally the receptacle is gently lowered into a vessel of cold water, so that the paraffin is cooled quickly, which prevents its crystallizing, but it cannot be entirely immersed until the paraffin has solidified over the top. When cold, the cake may be cut up into blocks of convenient size which are ready for cutting (Sec. 196). Material that is perfectly imbedded will be preserved indefinitely in a form that gives no further trouble, and for this reason it is often desirable to run material into paraffin instead of keeping it in alcohol.

SECTIONING

194. Free-hand sectioning. Free-hand sections are, as a rule, sufficiently satisfactory for general studies of the tissues of spermatophytes and pteridophytes. The technique is as follows. The object is held between the thumb and finger of the left hand, or, if small or soft, it must be placed between two flat pieces of pith. The razor is held in the right hand and is drawn across the object with the edge towards the operator and the blade sliding on the forefinger of the left hand. There should be water on the upper edge of the

razor, and as the sections are cut they should slip into the water and float in it. When a number of sections have been cut, they may be removed with a brush to a watch glass of water. It is, of course, impossible to cut good sections with a dull razor.

A small hand or table microtome is frequently of great assistance, taking the place of free-hand sectioning. The object is held between pith in an adjustable clamp, and the razor slides over a glass plate. A large number of sections, sufficient to supply a class, may thus be easily cut from such an object as a piece of stem or leaf.

Some methods of staining free-hand sections have been outlined in Secs. 183–186. Those of preserved material in alcohol require no further treatment before being placed in the stain, but sections of living material must be fixed before they can be satisfactorily stained. If the tissue is firm, the simplest method is to place the sections directly into absolute alcohol, when after an hour or so they may be stained. If, however, the tissue is delicate, or the cells contain much protoplasm, it is best to fix in medium chrom-acetic acid (Sec. 172) for two to twelve hours, washing for an hour or more in several changes of water. Such sections may be stained at once or run up into alcohol.

195. Sectioning in celloidin. As previously stated (Sec. 190), very firm and hard tissues such as characterize the sporophyte generation of pteridophytes and spermatophytes cannot be cut in paraffin. Exact work is frequently only possible through sections cut in celloidin. Furthermore, large sections of stems, roots, etc., can only be cut by this method. The technique is, however, somewhat long, and for the purposes of general studies free-hand sections, or those cut on a hand microtome, are likely to prove sufficiently satisfactory. A detailed account of the celloidin method as employed in botany is given by Plowman, *Botanical Gazette*, Vol. XXXVII, p. 456, 1904; and in Chamberlain's *Methods of Histology*.

196. Sectioning in paraffin. Sectioning in paraffin is only possible for structures of reasonably soft tissue and not very large. The advantages of the method are that the sections may be cut much thinner than in celloidin or free-hand, that they may easily be arranged serially, and that they may be stained with greater precision. The method is very generally applicable throughout the thallophytes and bryophytes and for the gametophyte generation of the pteridophytes and spermatophytes, together with the softer tissues of many organs and developing structures of the sporophyte generation of the latter groups.

Paraffin material is cut on a microtome. The most convenient instrument is the rotary microtome of the Minot type, of which there are several forms on the market. The sliding microtome of the Jung-Thoma type is also excellent, and while not so rapid as the rotary is sometimes more accurate for the most exact work.

The material, imbedded in the paraffin cake, is cut out in a small block, which is fastened by heat to a metal holder for the rotary microtome or to small wooden holders for the sliding ones. The block should be arranged so that it will be cut as nearly as possible in the correct plane. The paraffin is then trimmed around the object so that the cutting edge is square or rectangular, with parallel edges. The block is then adjusted by a mechanism so that the face which is to strike the knife is exactly parallel to its edge and so that the object will be cut in the desired plane.

Cutting in paraffin is not successful unless the sections run off the knife edge in an unbroken ribbon. There are a number of conditions necessary to obtain this result. The knife must be very sharp and the edge without nicks (at least where the cutting is done), which will split the ribbon lengthwise. It is useless to attempt to cut with a poor or dull knife. If a ribbon after running smoothly begins to split or show conspicuous lines, draw the finger upwards along the edge of the knife. The difficulty may have been caused by some hard particle lodged against the edge, which is thus removed. The edge of the knife must be clean; grease or paraffin may be removed with xylol applied by a brush or with a soft rag.

The ribbon should run straight. If it begins to curve, trim the block unevenly so that it will come off the knife straight; a curved ribbon is generally due to differences in the texture of the two sides of the object. Sometimes sections roll up or fail to stick together in a ribbon. This generally means that the paraffin is too hard for the temperature of the room. The cutting must be done in a warmer room or the material reimbedded in a softer paraffin. Cutting the sections in the sunshine of a window instead of in the shade will often remedy the difficulty. A more frequent difficulty is a crushing of the sections together. This means either that the knife is not sharp, that its edge is not perfectly clean, or that the paraffin is too soft for the temperature of the room. If the paraffin is too soft (as is commonly true in summer temperatures), the block and knife may be cooled in ice water or the material reimbedded in harder paraffin. It is time wasted to attempt to cut when the ribbon is not running smoothly; find out where the trouble lies and remedy it.

The finest quality of hone is necessary for sharpening microtome knives, and it should never be used for any other purpose. The Belgian stones are the best. There are also some good carborundum hones. The razor is gently passed back and forth on the hone with the edge forward, and stropping is not necessary or desirable if the hone is of the best quality. Soapy water is one of the best lubricants of the hone.

Sections are best cut from 7–10 micromillimeters thick for general histological work, but must be cut 5 or less for the finest details of protoplasmic structure. A micromillimeter (also called a micron) is a thousandth of a

millimeter. As the ribbon comes off the microtome knife it is removed in convenient lengths and laid in a series from left to right on a clean piece of paper. The ribbons may be kept indefinitely under a bell jar, but they are best mounted as soon as convenient, since they will collect some dust no matter what precautions are taken.

The ribbons are made to adhere to the slide with a fixative of the following formula (Mayer's albumen fixative):

white of egg	50 cc.
glycerin	50 cc.
sodium salicylate	1 gram

Mix well and filter. The sodium salicylate is an antiseptic and the fixative will keep for several months. Place a very small drop on the slide and with the tip of the little finger spread the thinnest film that can be laid on evenly over it. Then cover the film of fixative with water and place the ribbons cut to the proper lengths upon the water, arranged as desired. Warm the water gently over a flame; the paraffin will soften and the ribbons will expand and become perfectly smooth. The paraffin should not be allowed to melt. Drain the water off carefully and arrange the ribbons, which will now lie in a film of water, over the fixative. Put the slide aside to dry. It is frequently convenient to warm the ribbons in the water by placing the slide on the top of the paraffin bath and then to dry the slide in the same way, protected from too much heat by several thicknesses of blotting paper.

The preparations are not ready for staining on the slide until perfectly dry. They may be kept thus indefinitely, but it is best to stain soon, since the surface of the ribbons will inevitably collect dust. A great saving of time can be secured by preparing a number of slides at a time and carrying them simultaneously through the above processes and those of staining on the slide.

STAINING ON THE SLIDE

197. Preparation for staining on the slide. The dry slides with the ribbons adhering to the fixative may be placed in the bath to melt the paraffin, or they may be gently heated over a flame (with the ribbon side up), a process which must be managed carefully so as not to scorch the sections. The slide is then placed upright in a well of xylol (Stender dishes are convenient), which should not be near the flame. The xylol will dissolve the melted paraffin in a minute or so. The slide is then taken out of the well (the under side wiped off) and either placed in a well of 95% alcohol or a stream of alcohol is run over it from a pipette or wash bottle. The slide is now ready to be placed in the staining wells, of which there are various forms, but Stender dishes are satisfactory.

198. Bleaching after osmic acid. Material fixed in chrom-osmo-acetic acids (Flemming's fluids) is always blackened by the osmic acid. This blackening must be chiefly or wholly removed before staining. Microtome sections, after the solution of the paraffin with xylol and rinsing in 95% alcohol (Sec. 197), are placed in wells of 5–10% hydrogen peroxide in 70% alcohol. The bleaching is generally effected in an hour or less, but may require longer. Stronger solutions of hydrogen peroxide can be used if necessary, but it is safer to employ them weak. As soon as the gray or black tint is removed the slide is rinsed in 95% alcohol and is then ready for the stain. Small objects which are not to be sectioned (such as filamentous algæ) are treated in the same manner in watch glasses.

199. Staining on the slide. The best stains for the details of protoplasm are iron-alum hæmatoxylin, or safranin followed by gentian violet. Perhaps the most successful combination is safranin, gentian violet, and orange G, — a combination known as Flemming's triple stain, the use of which is, however, one of the most difficult of the staining methods. Delafield's hæmatoxylin, and safranin followed by Delafield's hæmatoxylin, are among the best general stains for tissues, and methyl green followed by fuchsin is also good.

A. *Iron-alum hæmatoxylin.* This method follows the same outline as is given in Sec. 182 for staining in bulk. The slide is taken from 95% alcohol, dipped in 35%, and placed in iron alum (best used in 1% solution) for from two to four hours; it is then rinsed in distilled water and left in hæmatoxylin from four to eight hours or over night. From the hæmatoxylin it is returned to the iron alum to extract the stain, and this process must be watched with care, the preparation being examined from time to time under the microscope. At the proper point of extraction the slide is placed in a large dish of tap water, where it must remain for fifteen minutes or more. It is then dipped in 35% alcohol (if desired) and left two or three minutes in a well of 95% alcohol (it may remain indefinitely in the 95%). Finally the slide is taken from the 95% alcohol, drained, and some absolute alcohol is poured over the sections from a small bottle and rapidly drained off, and the slide placed as soon as possible in a well of xylol (clove oil should never be used). The preparation must remain in the xylol half an hour or more, since the xylol and absolute alcohol do not mix rapidly, after which it is removed, the superfluous xylol drained off, and the sections mounted in balsam. Minute bubbles on the slide after having been in xylol indicate that the dehydration has not been perfect, and they must be removed by absolute alcohol and the slide again placed in xylol.

B. *Iron-alum hæmatoxylin* and *safranin.* The hæmatoxylin stain may be extracted by iron alum until it remains practically in the nucleus alone.

Then after passing through tap water it may be placed in a solution of safranin (Sec. 184) until the protoplasm and cell walls are slightly stained, after which it is carried through absolute alcohol into xylol.

C. *Delafield's hæmatoxylin.* This stain is excellent for tissues, since it colors the cell walls sharply, as is not done by iron-alum hæmatoxylin. Follow the outline given in Sec. 183.

D. *Safranin, gentian violet,* and *orange G.* It is not necessary to use the orange G in this combination, known as Flemming's triple stain, but the best results have been obtained with it. The technique of this method of staining is difficult, but it gives perhaps the most effective staining for the study of protoplasmic structure, especially during nuclear division. It is impossible to give more than a general programme of the method, since the time limits necessary to obtain satisfactory results vary with different material and must be tested experimentally.

The slide is transferred from 95% alcohol to a well of safranin. The alcoholic solution mixed with an equal part of water is good, as is also anilin safranin (Sec. 184). After remaining in safranin from four to twenty-four hours (over night is generally convenient), the slide is placed in 50% alcohol and the stain extracted until it remains in the nucleolus and chromatin alone. Acid alcohol (Sec. 184) may be used to extract the stain more rapidly, but it is generally not necessary.

The preparation is then placed in a well of gentian violet. A saturated aqueous solution is good, or a 1% solution is generally strong enough. The slide is left in gentian violet as short a time as possible to obtain good results, and this can only be determined by trial. Sometimes merely dipping it in the stain is sufficient; other material may require a number of seconds, or even minutes. On removal from gentian violet the slide is drained and rinsed in 50% alcohol, and then absolute alcohol is poured over the sections, followed by a few drops of oil of cloves placed in the center of the preparation. The oil of cloves may be replaced with cedar oil or xylol to avoid possible fading of the stain.

The secret of success with gentian violet is not to stain the nucleolus and chromatin so deeply that the stain will not wash out. They should be left red and the other protoplasmic structures blue. If the nucleolus and chromatin come out blue, the slide has been left too long in gentian violet. In our practice the best results have come with very short treatment in strong gentian violet (frequently only a dip, or a few seconds timed by the watch), followed directly by absolute alcohol. The oil of cloves may be depended upon to remove much of the gentian violet. But, as previously stated, material differs very greatly in its reaction to gentian violet, and each subject requires experimentation and a critical

examination at various stages in the process of staining, to correct errors. The commonest mistake is to overstain with gentian violet.

Should orange G be brought into the combination, the slide after removal from gentian violet is rinsed in water and placed in a 1% aqueous solution or a dilution of this strength. It is left from ten to thirty seconds in this stain and then treated with absolute alcohol and cleared in oil of cloves as described above for the gentian violet. The orange G, if successfully used, will give a grayish tinge to the cytoplasm, while the spindle fibers (kinoplasm) will be blue and the nucleolus and chromatin red. The combination, when successful, is the most striking stain known for nuclear figures.

Slides should not be destroyed if the results are not up to expectations. If the protoplasm is uniformly blue, structures may still show fairly well, and if the stain is too weak, the balsam may be removed with xylol and the slide stained again. It is not necessary to use orange G, and in our own practice this stain is generally omitted.

E. *Safranin* and *Delafield's hæmatoxylin.* This combination, applied as outlined in Sec. 185, is excellent for the staining of tissues, without much regard for the details of protoplasmic structure, such as nuclear figures, etc.

F. *Other stains.* Other anilin dyes besides safranin and gentian violet are frequently used in staining on the slide. Fuchsin (Sec. 186, A), methyl green followed by fuchsin (Sec. 186, B), and erythrosin after hæmatoxylin, or blue or green anilin stains (Sec. 186, C) give good results for the study of tissues. They are especially satisfactory for the differentiation of structure in fibro-vascular bundles.

CULTURE METHODS

THE CULTURE OF ALGÆ

200. Culture in aquaria. The most convenient forms of aquaria are shallow glass dishes eight to ten inches wide, battery jars six to eight inches wide, or other large glass receptacles. These should be loosely covered with pieces of heavy glass to keep out the dust, and should not be filled more than two thirds full of water. It is not generally necessary to aërate the water, and cultures should rather be left to themselves to grow the forms with which they are stocked, or to develop whatever types may appear. It is always interesting, and frequently surprising, to see what growths will develop of their own accord in aquaria. There should be no metal in contact with the water of aquaria, and copper is especially poisonous.

Some algæ, such as the water net, *Hydrodictyon*, species of *Œdogonium*, *Coleochæte*, *Chara*, *Oscillatoria*, and numerous one-celled forms grow readily in aquaria. Other types are more difficult to cultivate, as *Spirogyra* and other pond scums, and it is almost impossible to keep the red or the brown marine algæ alive for any length of time. Terrestrial species of *Vaucheria* frequently grow luxuriantly over the earth of the flowerpots in greenhouses.

Algæ are more likely to survive in aquaria when kept in the water of the ponds and ditches from which they came. Such water may be filtered, and the aquaria should be stocked with only a small amount of the algæ. It is not desirable to have animals such as snails or crustacea in the aquaria, for there is almost sure to be present a sufficient quantity of microscopic forms to preserve a balance of animal and plant life. The aquaria are best placed outside the room on window ledges, except in freezing weather, and they should have very little, if any, direct sunlight.

A. *Knop's solution.* There are a number of culture solutions. One of the best known is that of Knop, made as follows :

potassium nitrate	1 gram
potassium phosphate	1 gram
magnesium sulphate	1 gram

These three salts are dissolved in one half liter of rain water or fresh tap water, that is tap water which has not been standing in metal pipes. To this is added a solution of 4 grams calcium nitrate in one half liter of similar water. There will be formed an insoluble precipitate of

calcium phosphate which is left in the fluid. This makes a .7% solution of the salts, which is too strong for general purposes. It is better diluted with an equal quantity of water (making a .35% solution), or, for delicate algæ, with two liters of water (making what is approximately a .2% solution of the salts). Algæ are placed directly in this culture solution, and many of them do well in it.

B. *Moore's solution.* Moore reports that the following solution (which is a modification of one of Beyerinck's) is much more satisfactory than that of Knop:

ammonium nitrate	.5 gram
potassium phosphate	.2 gram
magnesium sulphate	.2 gram
calcium chloride	.1 gram
iron sulphate	trace

These salts are dissolved in a liter of water. For blue-green algæ the amount of ammonium nitrate should be doubled, and 1-2% of glucose may be added with benefit.

C. *Cane sugar.* A 2-4% solution of cane sugar is an important fluid, since some algæ — as *Vaucheria*, *Hydrodictyon*, and *Spirogyra* — will generally fruit after a few days when transferred to it from pond water or cultures solutions and exposed to bright light or moderate sunshine.

201. Cultures on agar-agar. Pure cultures of unicellular algæ may be grown and isolated on agar-agar mixed with a nutrient solution. Moore recommends his modified Beyerinck's solution (Sec. 200, B), with double the amount of ammonium nitrate and 2% of glucose. To a liter of this solution (heated to boiling) 5 grams of agar is added, and after its liquefaction the fluid is poured into small Erlenmeyer flasks of 100 cc. capacity, or other small dishes which may be tightly covered; on cooling, the liquid will stiffen to a moist jelly. Pure cultures may be easily established in such vessels, and if protected from drying will flourish for years.

THE CULTURE OF FUNGI

202. Cultures in moist chambers. Fungi will grow in abundance upon a great variety of substances when kept damp in moist chambers. The most convenient form of a large moist chamber is a rather low bell jar five to six inches high, set in a dish of water. The substance is placed on some support, such as a zinc rack, so that it is raised above the surface of the water, the evaporation of which keeps the air in the interior of the bell jar moist. It is well also to line the interior of the bell jar with moist filter paper in contact with the water below. Cultures upon large pieces of bread and cheese are

best arranged for in this manner. Smaller moist chambers may be made by placing filter paper above wet *Sphagnum* on the bottom of shallow glass dishes, such as crystallizing dishes, three or more inches high, covered by a piece of glass. The substance to be used as the substratum is placed on the filter paper, which is kept moist by the *Sphagnum*. Such chambers are well suited to cultures on small pieces of fruit or other vegetable matter, and on the dung of various animals. Cultures on horse dung are best made in larger dishes or under bell jars.

203. Pure cultures on potato agar. Most saprophytic fungi may be cultivated on potato agar, which is one of the simplest and most satisfactory of the culture media.

To make potato agar, pare two or three medium-sized potatoes, cut into thin slices, place in a stewpan, and cover with tap water. Allow the water to simmer gently for one half hour, or until the potatoes are soft but not disorganized. Do not let it boil. Strain the liquid, which should be as clear as possible. Add enough tap water to make one half liter, and place in a flask with 10 grams of agar-agar cut up in small pieces. Heat the flask in a steam sterilizer until the agar has melted and mixed with the culture fluid.

Clean about thirty test tubes (six inches long and three fourths of an inch across), rinse, drain, and dry. Fit cotton plugs of a good quality into the dry test tubes. They should enter the tubes at least an inch and project somewhat beyond. Place the tubes fitted with cotton plugs in a dry-air sterilizer and expose to a temperature of 140° C. for an hour; this will kill all spores of fungi, including bacteria, in the tubes or on the cotton. The tubes are best handled in a receptacle made of heavy wire netting, and of a size which will slip into the steam sterilizer.

Pour the melted potato agar into the test tubes by means of a funnel, removing the cotton plug carefully and holding between the fingers while filling each. The tubes should be filled up about one and one-half inches from the bottom. It is very important that no agar become smeared around the top of the test tube where the plug is inserted. The filled tubes, carefully plugged, are now sterilized twice a day (morning and night) on three successive days in the steam sterilizer for an hour each time. This is necessary to render the tubes free from bacteria, for the spores of bacteria are not generally killed by the temperature of 100° C., but they germinate quickly in the potato agar, and the vegetative bacteria produced by them are then killed by that temperature.

After the sterilization of the third day the tubes are taken out and laid on a table inclined against a board so that the surface of the hot fluid agar runs three or four inches up the sides of the tubes. As they cool, the agar stiffens in this position, forming a long, slanting surface in each tube, which is now ready for inoculation.

Transfers of spores are made to the tubes by means of a piece of stiff platinum wire about two inches long, set in the end of a glass rod when melted in a flame. The lower end of the rod and the wire are sterilized in a flame and the point of the wire is touched to a single spore-bearing hypha in a culture. The cotton plug is then carefully removed from a tube and held between the fingers while the point of the wire is drawn over the surface of the agar. It is best that the tube be held inverted while being inoculated so that dust may not enter. The plug is replaced quickly and the inoculated tube is laid aside to await developments. If but a single spore-bearing filament has been touched, the culture will probably be pure. Should an impure culture develop, transfers may generally be made from it to another tube. Tubes with cultures may be prevented from drying out too rapidly by cutting off the tops of the cotton plugs and coating the ends of the tubes with paraffin.

Potato agar may be poured into Petri dishes and sterilized in the same manner as the test tubes. Such dishes are excellent for studies upon the bacteria as outlined in Sec. 100.

204. Hanging-drop cultures. These are used for the study of germinating spores, pollen grains, and other subjects. The spores are placed in a drop of the culture fluid on a cover glass, which is then arranged so that the drop hangs down from the lower side in a small moist chamber on a slide. The chamber may be made of a ring of glass cemented on the slide with wax or gold size (Van Tieghem cell), over which the cover glass fits and is sealed with vaseline. A little distilled water in the bottom of the chamber will save some evaporation from the drop of the culture fluid.

A more temporary but also effective chamber may be made by cutting a square hole about one half inch in diameter in the center of a piece of cardboard one inch wide, one and one-half inches long, and one eighth of an inch or more thick. The cardboard is boiled and pressed closely on the slide. The culture drop is then placed in the center of a cover glass an inch square or slightly smaller. This is inverted over the hole in the cardboard so that the culture drop hangs down in the center and the cover glass is then pressed closely against its wet surface. Water is added from time to time to the edge of the cardboard to keep it moist, and the slide when not being studied may be placed in a moist chamber, which will hinder the cardboard from drying rapidly.

The culture fluids vary with the subject. Boiled decoctions of horse dung are good for the germination of many fungal spores. Decoctions of decayed wood are used for the spores of slime molds. Solutions of sugar (3–30% in tap water) are employed in the germination of pollen grains (see Experiment XLII); 1.5% of gelatin may sometimes be added to advantage to the sugar solutions. Spores of mosses and ferns germinate readily in sweetened water.

THE CULTURE OF LIVERWORTS AND MOSSES

205. The culture of liverworts. Aquatic liverworts, such as *Ricciocarpus natans* and some species of *Riccia*, will sometimes grow fairly well in large glass aquaria, but they must have pure air and considerable water. They will do much better in tanks or cement basins in greenhouses. The terrestrial liverworts, such as *Marchantia*, *Lunularia*, *Conocephalus*, and related types, grow readily on damp soil in greenhouses or in large vessels covered with glass. These forms and various mosses are frequently present in ill-kept greenhouses. They will not do well in very bright light, preferring shaded situations, and must have abundant moisture in the earth. If convenient, it is well to cultivate them on soil from their habitats.

206. The culture of protonemata. Moss spores germinate readily, and it is not difficult to obtain luxuriant cultures of protonemata. These frequently appear over the surface of earth in flowerpots in greenhouses, and then somewhat resemble the more common growths of *Vaucheria*. Cultures of the spores are conveniently made in bulb pots or other wide pots set in saucers of water. After filling the pot with earth to an inch from the top, it is well to heat it for two or three hours in a steam sterilizer to kill fungi which might be troublesome, but this is not absolutely necessary.

The spores of many of the common mosses of the fields will grow readily, but species of *Funaria* are especially satisfactory. The spore cases may be crushed over a sheet of paper to remove the spores, which are then gently blown over the surface of the earth. The top of the pot is covered with a piece of glass and the culture is watered from the saucer below. The earth should be merely moist, not wet, for too much moisture may result in the death of the culture by "damping off" from the growth of fungi. The earth will shortly become covered with a growth of green filaments. After two or three weeks, buds will be developed, followed by the appearance of the leafy moss plants.

207. Moss cultures. A thick growth of leafy moss plants generally arises from the protonemata as described above. These plants will in time develop sexual organs, the antheridial plants being easily distinguished by the rosette of expanded leaves around the yellow or orange-colored clusters of antheridia. The archegonia will not be fertilized if the culture is watered entirely from the saucer below. When sporophytes are desired the culture must be flooded with water, first closing with a cork the opening in the pot below. It should remain flooded for an hour or more, after which the water may be allowed to run off. All ripe archegonia and antheridia will have opened, and the eggs, having been fertilized, will develop sporophytes. By successively flooding the culture at intervals, sporophytes in various stages of development may be obtained.

THE CULTURE OF FERNS

208. The culture of fern prothallia. Fern prothallia may be cultivated even more easily than moss protonemata. The method is essentially the same. The spores of common greenhouse ferns will germinate readily, but the prothallia of some present abnormalities due to apogamy (*Principles*, Sec. 311), so it is better to sow the spores of some of the wild ferns, such as *Pteris aquilina*, species of *Onoclea*, *Aspidium*, *Polypodium*, etc. Such spores generally retain their vitality for a year or more. The spores of *Osmunda*, on the contrary, and also those of *Equisetum* live only a few days and must be sown at once at maturity, but then give very luxuriant cultures.

The spores, crushed out of their sporangia on a piece of paper, are blown over the surface of earth in bulb pots or shallow dishes. These are covered with glass and the pot is set in a saucer and watered from below. The earth in the pot may be sterilized with advantage (Sec. 206). The culture should not be kept too moist, for there is danger of the prothallia damping off. Prothallia will begin to develop antheridia in three or four weeks and will be full-grown in six weeks. Care should be taken not to sow the spores too thickly, at least in portions of the dish, for crowded growths of prothallia remain dwarf and only develop antheridia.

Growths of young fern sporophytes are obtained by flooding a culture of mature fern prothallia for an hour or more, closing the bottom of the pot temporarily as described in Sec. 207. In a few weeks the first leaves of the young fern plants will appear, growing up in the notch of the large fern prothallia.

209. Water ferns. The floating water ferns *Salvinia* and *Azolla*, like the floating liverworts (Sec. 205), will grow in glass vessels if they have pure air and plenty of water, but they do much better in large tanks or cement basins in greenhouses. *Marsilia* is hardy and grows well in tanks or basins. *Salvinia* and *Azolla* may be kept thus over winter, and in the spring, when placed in ponds out of doors, will generally do well. *Marsilia* is easily introduced into ponds, where it forms thick growths in shallow water. *Salvinia* is not uncommon under cultivation in water-lily ponds of city parks.

THE CULTURE OF SEED PLANTS

210. Directions for the culture of seed plants. It is hardly worth while to give an account of the manner in which such seed plants as are needed for studies and demonstrations are best cultivated. The advice of a competent florist will be found more helpful than any set of printed directions. *Das Pflanzenmaterial für den botanischen Unterricht*, by Dr. P. Esser, I. Teil, Cologne, 1903, gives much useful information. Its price is Marks 3.20.

MATERIAL, APPARATUS, AND SUPPLIES

LISTS OF PREPARATIONS FOR THE MICROSCOPE

211. Slides of value in studies on the plant cell.

Spirogyra. To show the nucleus: filaments fixed in weak chrom-acetic acid (Sec. 172, A), stained in iron-alum hæmatoxylin (Sec. 182), mounted in glycerin (Sec. 188); or stained in Magdala red and anilin blue and mounted in Venetian turpentine (Chamberlain, *Methods of Histology*, p. 81, 1905).

Root tip of onion or hyacinth. For the study of cell and nuclear division: fixed in medium chrom-acetic (Sec. 172, B), or weak Flemming (Sec. 173, A), sectioned in paraffin from five to seven micromillimeters thick, stained with safranin and gentian violet (Sec. 199, D).

Pollen mother cells of the lily or related types. Also excellent subjects for the study of cell and nuclear division (see *Lilium* in next section).

212. Slides of value in type studies. The following list of preparations is merely suggestive; many other subjects may be added. It is a mistake to suppose that permanent preparations are necessary for type studies. They will, however, at times be of great assistance. The accumulation of class preparations requires time and patience and their proper use demands judgment. There is much danger in giving slides to students before they are prepared to understand from studies on living or preserved material the topography and significance of the structures shown in the preparations.

Volvox. Whole colonies, safranin or iron-alum hæmatoxylin, carried with care into balsam (Sec. 187), the cover glass supported by two strips of paper to prevent crushing.

Ulothrix, or other alga, forming zoöspores. Iron-alum hæmatoxylin and safranin (Sec. 182), carried with care into balsam.

Œdogonium. Iron-alum hæmatoxylin and safranin, glycerin, or carried with care into balsam.

Coleochæte. Fruiting thallus, Delafield's hæmatoxylin (Sec. 183), balsam.

Fucus. Paraffin sections of oögonial conceptacles to show histological details, safranin and gentian violet, or D.'s hæmatoxylin.

Sporodinia or Rhizopus. Zygospores with mycelium slightly stained with D.'s hæmatoxylin, glycerin, or balsam.

Albugo. Paraffin sections of (1) blisters, D.'s hæmatoxylin; (2) tissue with oögonia, safranin and gentian violet.

Peziza or other cup fungus. Paraffin sections of fruiting surface, safranin and gentian violet.

Puccinia. Paraffin sections (1) across sori of teleutospores and uredospores ; (2) cluster cup on barberry, D.'s hæmatoxylin.

Coprinus or other gill fungus. Paraffin sections of gills, cut rather thick, showing basidia with spores, D.'s hæmatoxylin.

Marchantia. Paraffin sections (1) of thallus, safranin and D.'s hæmatoxylin (Sec. 185) ; (2) antheridial receptacles, D.'s hæmatoxylin ; (3) archegonial receptacles for archegonia and sporophytes, safranin and D.'s hæmatoxylin.

Porella. Paraffin lengthwise sections (1) of antheridial branches, D.'s hæmatoxylin ; (2) archegonial branches for sporophytes, safranin and D.'s hæmatoxylin.

Anthoceros. Paraffin lengthwise sections of medium-sized sporophytes attached to small pieces of the gametophytes, safranin and D.'s hæmatoxylin.

Funaria, Mnium, or Atrichum. Paraffin sections (1) of the tips of antheridial and archegonial plants ; (2) medium-sized spore cases for spore-bearing tissue, D.'s hæmatoxylin.

Webbera. Protonema, common in greenhouses, frequently forms buds in great numbers, safranin, carried with care into balsam.

Aspidium. Paraffin sections of sorus, D.'s hæmatoxylin.

Pteris aquilina. (1) Cross and lengthwise sections of rhizome (Sec. 194) mounted on the same slide, safranin and D.'s hæmatoxylin.

Pteris or other fern. Paraffin lengthwise sections of (1) root tip ; (2) large prothallia to show archegonia and occasional developing sporophytes, safranin and D.'s hæmatoxylin.

Pinus. (1) Cross sections of needle, safranin and D.'s hæmatoxylin ; (2) Sections of wood, cross, radial, and tangential, mounted on the same slide, safranin and D.'s hæmatoxylin, or methyl green and fuchsin (Sec. 186, B) ; (3) paraffin lengthwise sections of ovules from a year-old cone, safranin and gentian violet or safranin and D.'s hæmatoxylin.

Lilium or related types. (1) Paraffin cross and lengthwise sections of anthers, showing pollen mother cells in stages of nuclear and cell division, also sections of mature anthers ; (2) paraffin cross sections of ovule cases of various ages to show the development and structure of the embryo sac and ovule ; (3) double fertilization in embryo sac ; (4) development of embryo and endosperm ; all stained with safranin and gentian violet, or safranin, gentian violet, and orange G (Sec. 199, D).

Sambucus. Paraffin cross sections of mature anthers to show gametophyte nuclei in the pollen grain, safranin and gentian violet.

Capsella. (1) Paraffin lengthwise sections of tip of growing raceme to show stages in the development of the flowers, D.'s hæmatoxylin ; (2) paraffin

lengthwise sections of ovules after fertilization, showing embryo in embryo sac, safranin and D.'s hæmatoxylin.

213. Slides of value in histological studies on the seed plants.

ROOTS

Corn root tip. Lengthwise section, showing plerome, periblem, and dermatogen, safranin and Delafield's hæmatoxylin.

Tradescantia root tip. Lengthwise section, showing general root structure and nuclear division, safranin and gentian violet.

Smilax root. Cross section, showing cortex, endodermis, and radial bundles, safranin and D.'s hæmatoxylin.

STEMS

Hippuris shoot. Lengthwise section of stem apex, showing meristem, origin of lateral shoots and leaves, safranin and D.'s hæmatoxylin.

Indian corn stem. Lengthwise section, showing sieve tubes, companion cells, annular tracheids and sclerenchyma, safranin and D.'s hæmatoxylin.

Pumpkin stem. Lengthwise section, showing sieve tubes and companion cells and vessels, safranin and D.'s hæmatoxylin.

Menispermum stem. Cross section, showing separate bundles of a climbing stem with large vessels for conducting water, safranin and D.'s hæmatoxylin.

Brasenia stem. Cross section, showing typical structure of an aquatic stem with large air cavities and scanty vascular system, D.'s hæmatoxylin.

Dodder on golden-rod stem. Cross section, showing penetration of stem tissues by haustoria, safranin and D.'s hæmatoxylin.

LEAVES

Cycas revoluta. Cross section, extremely xerophytic leaf structure with thick cuticle, highly developed palisades, and depressed stomata, safranin and D.'s hæmatoxylin.

Peperomia. Cross section, typical of water storage in the leaf outside of the photosynthetic tissue, D.'s hæmatoxylin.

Silphium laciniatum. Cross section, vertical leaf with a palisade layer near each surface, D.'s hæmatoxylin.

Potamogeton. Cross section, submerged leaf with thin epidermis, no stomata nor palisades, large air cavities and scanty vascular system, D.'s hæmatoxylin.

Water buttercup. Cross sections (1) of aërial leaf; (2) submerged leaf, D.'s hæmatoxylin.

SUGGESTIONS ON MATERIAL FOR THE STUDY OF PLANT HISTOLOGY

214. Histological material of the seed plants.

Air passages. Rootstock of sweet flag (*Acorus*); stem of *Juncus*, *Myriophyllum*, *Scirpus;* leafstalks and flower stalks of pond lily and of *Nuphar.*

Aleurone grains. Seeds of almond, Brazil nut, castor bean, and nutmeg.

Bast fibers. Stem of flax, of hemp, of linden (young twig) ; leaf of *Carludovica*, *esparto* grass, palm, pineapple.

Bundles, closed. Stem of asparagus, corn, green brier (*Smilax*) ; flower stalks or leaves of *Yucca filamentosa ;* petioles of fan palm leaves (cut if necessary from the handle of a palm-leaf fan).

Bundles, open. Young stems of *Aristolochia*, *Begonia*, *Clematis*, evening primrose, *Menispermum ;* stems of sunflowers or other large composites.

Cambium. See *Bundles, open.*

Cambium, cork (*phellogen*). Young twigs of *Abutilon* (greenhouse species) and of elder.

Central cylinder of root. Roots from bulb of hyacinth or onion ; roots of *Acorus*, *Actæa*, *Smilax*, *Veratrum.*

Chlorophyll bodies. Best seen in leaves of moss, as *Funaria* or *Mnium*, or in fern prothallia ; thin sections of any green leaves or upper epidermis torn off, with the tops of the palisade cells attached ; large and distinct in leaves or bracts of pineapple.

Chromatophores or chromoplasts. Surface sections from sepals of *Tropæolum*, pulp of fruit of *Crataegus*, asparagus, or rose, root of carrot ; many algæ, such as *Spirogyra*, *Ulothrix*, desmids, diatoms, *Ectocarpus*, *Nemalion*, etc.

Collenchyma. Young stems of *Aristolochia Sipho ;* stems of begonias, *Salvia*, and most *Umbelliferæ.*

Cork. Young twigs of *Ailanthus*, sweet gum (*Liquidambar*), cherry, currant, *Cytisus Laburnum*, *Euonymus alatus ;* ordinary bottle corks (from *Quercus Suber*) ; cortex of potato tuber.

Cuticle. Leaves of *Agave*, *Aloe*, *Cycas revoluta*, *Ficus elastica*, *Yucca filamentosa.*

Embryo sac. Lily, buttercup, and their allies.

Epidermis. See under *Cuticle.* Also thin and easily peeled epidermis of iris, lily, hyacinth.

Fertilization and development of embryo. Young fruit of *Capsella*, *Monotropa*, *Pyrola*, *Veronica*, lily, buttercup.

Growing point. Tip of stem of *Myriophyllum* or *Hippuris ;* buds of lilac, *Cratægus*, *Viburnum.*

Hairs and scales. Glandular hairs, "geraniums," most *Labiatæ*, sundew, tomato; ordinary unbranched hairs, leaves and stems of most *Borraginaceæ*, *Gnaphalium*, seeds of cotton; scale-like hairs, leaves of *Elæagnus*, olive, *Shepherdia;* star-shaped hairs, leaves of *Matthiola;* stinging hairs, stem of nettle; T-shaped hairs, leaves and stems of *Artemisias;* branched hairs of mullein.

Laticiferous tissue. Root of chicory, of dandelion; stem of *Euphorbia splendens*, of lettuce; bract of *Ficus elastica.*

Leaf structure. Hydrophytic: *Elodea*, *Potamogeton*, submerged leaves of *Sagittaria;* mesophytic: most deciduous trees and shrubs; xerophytic: see under *Cuticle*, — also bearberry, crowberry, *Elæagnus*, holly, oleander, mistletoe, olive.

Lenticels. Young twigs of birch, cherry, elder, and sumac.

Leucoplasts. Pseudo-bulbs of *Phajus grandifolius*, rootstocks of *Iris germanica.*

Nuclear division (mitosis). Pollen mother cells of lily and its allies; cells of root tip of onion or hyacinth.

Nuclei. Epidermal cells of many leaves; growing points of roots or stems; hairs of roots, stamen hairs of *Tradescantia*, hairs of stem of cucumber; internodes of *Tradescantia;* bulb scale of onion; pollen mother cells of lily and its allies.

Oil and resin glands. Aments of the hop; hairs and emergences on leaves of any aromatic *Labiatæ;* leaves of *Eucalyptus globulus*, of *Ruta;* rind of lemon or orange.

Oil as reserve material. Oily seeds, as almonds, Brazil nuts, cacao seeds, castor beans, peanuts, squash seeds, sunflower seeds.

Palisade cells. Leaves of beech, *Cycas*, English ivy, *Ficus elastica*, holly, Japan quince, mistletoe, oleander, poplar, privet, willow, yucca.

Pollen tubes. Pollen of snowdrop (*Leucojum*), sweet pea, *Tropæolum*, tulip.

Root, dicotyledonous. Beans and other *Leguminosæ*, *Compositæ*, grapevine, primrose, *Ranunculus;* very young roots of most hardwood shrubs and trees.

Root, monocotyledonous. See *Central cylinder of root.* Also asparagus, *Aspidistra*, corn and other large grasses, *Iris*, sedges, *Smilax.*

Rootcap. Monocotyledonous: corn and other grasses, *Iris;* dicotyledonous: bean, pea, sunflower, *Tradescantia* grown in water.

Root hairs. Most roots of very young seedlings from seeds sprouted on wet paper in a slightly damp atmosphere, *Tradescantia* grown in water.

Sclerenchyma fibers. See *Bast fibers* and *Wood fibers.*

Secondary thickening. Twigs two years old and more of coniferous and of hardwood trees.

Seeds. With endosperm : buckwheat, castor bean, four-o'clock, all grasses, morning-glory, honey locust ; without endosperm[1] : all *Cucurbitaceæ*, most *Leguminosæ*, all *Rosaceæ*.

Sieve tubes. Stems of *Cucurbitaceæ*, young stems of grapevine.

Starch. Rootstocks of arrowroot (*Maranta*), of *Canna ;* seeds of beans, buckwheat, corn, oats, rice, wheat ; stems of *Euphorbias ;* tubers of potato.

Stem, dicotyledonous. See *Bundles, open.* Very young twigs of most hardwood shrubs and trees.

Stem, monocotyledonous. See *Bundles, closed.* Rootstocks of grasses and sedges ; stem of bamboo and of rattan.

Stomata. Leaves of *Aloe*, of *Crassulaceæ*, *Cycas revoluta*, *Ficus elastica*, *Iris*, *Liliaceæ*, oleander.

Stone cells. Allspice fruit, clove flower stalk, oak bark, pear-fruit stalk.

Tracheids. Wood of any coniferous shrub or tree.

Vessels. Leaf of banana ; peduncle of banana, of yucca ; root of *Acorus*, of *Iris ;* stem of corn, evening primrose, grapevine, rattan, *Ricinus*, sunflower.

Water-storage system. Leaves of *Agave*, *Aloe*, *Ficus elastica*, *Mesembryanthaceæ*, *Peperomia ;* stems of *Cactaceæ*.

Wood fibers. Fibrous hard wood, as alder, birch, hickory, linden, locust, magnolia, poplar (*Populus*).

Wood parenchyma. Wood of apple, bladder nut, hawthorn, linden, pear, red cedar, rose.

References. Strasburger-Hillhouse, 6 ; Strasburger, Noll, Schenck, Karsten, 1 ; Tschirch, 74.

APPARATUS FOR THE LABORATORY

215. Apparatus. The stocking of a laboratory with apparatus is a matter of time and experience, depending upon the character of the work given. The following list is therefore merely suggestive :

Aquarium jars. Large battery jars or museum jars are also good.

Balances, large, capacity $\frac{1}{10}$ gram to 2 kilograms, with weights.

Balances, small, capacity 1 milligram to 100 grams, with weights.

Battery jars, glass, quarts and gallons.

Bell jars from five to six inches high, and one or more tall ones.

Blotting paper.

Bottles, dropping.

Bottles, glass stoppered, assorted.

Bottles, ordinary wide mouthed, assorted.

Boxes, small, wooden, for germination experiments.

[1] I.e. with the reserve-material practically all contained in the embryo, even if traces of endosperm remain.

Camera lucida.
Chemical thermometers, registering 100° C. and above.
Clinostat.
Clock glasses.
Corks.
Crystallizing dishes.
Culture jars, large and small flat glass dishes.
Dishes, ordinary plates and saucers.
Evaporating dishes.
Eyepiece micrometer.
Flasks.
Flowerpots, ordinary, and bulb pots, with saucers.
Funnels, glass, assorted sizes.
Glass growing case (see Wardian case).
Graduated cylinders, 10, 100, 500 cc.
Hones, one rough for scalpels, one Belgian or carborundum for microtome knives.
Imbedding oven.
Lead, thin sheet.
Mason butter jars for preserved material.
Microtomes.
Museum jars, very useful but expensive.
Petri dishes.
Pipettes (medicine droppers).
Printing frames, photographic.
Printing paper, ordinary photographic and for blue prints.
Razor strops.
Retort stands.
Sand.
Sawdust.
Stage micrometer.
Stender dishes.
Sterilizer, steam.
Test tubes.
Thermostat.
Thistle tubes.
Tools, such as hammer, saw, file, screw-driver, pliers, monkey wrench, cork borers, etc.
Tubing, glass assorted and also rubber.
Tumblers.
Wardian case (glass growing case), Ganong, 7, p. 82.
Wash bottles.
Watch glasses, solid.

CHEMICALS FOR THE LABORATORY

216. The supply of necessary chemicals in a laboratory will depend upon the character of the work. This list is therefore merely suggestive.

Acids, commercial acetic, glacial acetic, chromic, hydrochloric, nitric, osmic, sulphuric.
Alcohol, commercial and denatured.
Alcohol, absolute.
Ammonia.
Ammonia sulphate of iron (iron alum).
Benzine.
Calcium nitrate.
Calcium oxide (quicklime).
Calcium sulphate (plaster of Paris).
Canada balsam.
Chloroform.
Chlorzinc iodine (see Sec. 169).
Ether.
Fehling's solution (see Sec. 170).
Ferric chloride.
Formalin.
Glycerin.
Grafting wax.
Hydrogen peroxide.
Iodine.
Magnesium sulphate.
Mercury.
Millon's reagent (see Sec. 170).
Oil of cloves, cedar oil, olive oil.
Paraffin, hard and soft.
Potassium chloride.
Potassium hydroxide (caustic potash), 5% and 15% solutions (see Sec. 169).
Potassium permanganate.
Potassium phosphate, acid.
Sodium chloride (common table salt and also c.p.).
Stains, acid fuchsin, eosin, erythrosin, gentian violet, hæmatoxylin, iodine, methyl green, orange G, phloroglucin, safranin (directions for making up these stains are given in Secs. 169, 170, 181–186).
Sugar, cane.
Vaseline.
Water, distilled.
Xylol.

DEALERS IN MATERIAL, APPARATUS, AND SUPPLIES

217. Material and slides. Plant material, both preserved and dried, and prepared slides for the microscope are offered by the following dealers, from whom price lists may be obtained.

A. Cambridge Botanical Supply Company, Cambridge, Mass. Preserved and living material, slides. See also Sec. 218, B, and Sec. 219, A.

B. Miss E. M. Drury, 45 Munroe Street, Roxbury (Boston), Mass. Slides and preserved material.

C. Marine Biological Laboratory (Botanical Supply Department), George M. Gray, Woods Hole, Mass. Preserved material of thallophytes and bryophytes, mounted sheets of marine algæ.

D. H. M. Phillips, 19 Warriner Avenue, Springfield, Mass. Plant material, especially fungi.

E. St. Louis Biological Laboratory, St. Louis, Mo. Slides and preserved material. See also Sec. 219, B.

F. Williams, Brown & Earle, 918 Chestnut Street, Philadelphia, Pa. Slides. See also Sec. 218, K.

G. Queen & Company, 1010 Chestnut Street, Philadelphia, Pa. Slides. See also Sec. 218, L.

218. Apparatus and supplies. Microscopes, physiological and other apparatus, glassware, general botanical supplies, etc., are sold by the dealers listed below from price lists which may be obtained from them.

A. Bausch & Lomb, Rochester, N.Y. Microscopes, apparatus especially for plant physiology, glassware, chemicals, and general supplies.

B. Cambridge Botanical Supply Company, Cambridge, Mass. Physiological apparatus, instruments, special botanical equipments, notebooks, general botanical supplies.

C. James T. Dougherty, 409 West 59th Street, New York City. Reichert microscopes, apparatus, glassware, and chemicals.

D. Eimer & Amend, 205 Third Avenue, New York City. Glassware, chemicals, and many instruments, general importers.

E. L. E. Knott Apparatus Company, Ashburton Place, Boston, Mass. Apparatus and supplies for general and special purposes.

F. Kny-Scheerer Company, 225 Fourth Avenue, New York City. Apparatus, chemicals, and supplies, importers.

G. Ernst Leitz, 30 East 18th Street, New York City. Microscopes, apparatus, glassware, chemicals, and general supplies.

H. Spencer Lens Company, Buffalo, N.Y. Microscopes, apparatus, glassware, chemicals, and supplies.

I. The Scientific Shop, 322 Dearborn Street, Chicago. Zeiss microscopes, special pieces of apparatus, photographic and projecting instruments.

J. Whitall, Tatum & Co., New York City. Glassware.

K. Williams, Brown & Earle, 918 Chestnut Street, Philadelphia, Pa. Beck microscopes, apparatus, glassware, and general supplies.

L. Queen & Company, 1010 Chestnut Street, Philadelphia, Pa. Microscopes, apparatus, glassware, and general supplies.

219. Lantern slides and charts. Lantern slides are sold singly or in sets by :

A. Cambridge Botanical Supply Company, Cambridge, Mass.

B. St. Louis Biological Laboratory, St. Louis, Mo.

There are a number of sets of charts on the market. The most complete are :

C. Kny. *Botanische Wandtafeln*, a series of 105 so far published, price 355 Marks. Paul Parey, Hedemannstrasse 10, Berlin, S.W.

D. Frank-Tschirch. *Wandtafeln für den Unterricht in der Pflanzenphysiologie*, a series of 60, price 180 Marks. Published also by Paul Parey (see above).

A new set of charts has recently been announced, which promises well.

E. Baur-Jahn. *Tabulæ Botanicæ*, to appear in series of five, 25 Marks a series. Published by Gebrüder Borntraeger, Dessauerstrasse 29, Berlin, S.W.

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APPENDIX

1. Laboratory notes. Much of the value of laboratory work must always depend upon the way in which the notebooks are inspected. Those with detached leaves, which may be assembled in some kind of binder, are to be preferred; and the instructor may certify his acceptance of the notes, page by page, either during the laboratory period or immediately afterward. In general, unsatisfactory notes should be rewritten from new studies of the material in question or (better) of equivalent forms.

Valuable data of any sort secured during the year's work should be preserved for subsequent use. In this way, for example, the optimum conditions for many of the phenomena of plant life may be ascertained by assembling the results of the best student observers. Duplicates of photographs and drawings may be kept in the botanical museum.

2. Students' preparations. In all cases in which the student finds it advisable to make for himself a set of preparations, e.g. of slides to illustrate any histological topic, such preparations should be treated as a part of his laboratory record. A duplicate series for the laboratory can usually be made with little extra effort. In the same way preserved material illustrating the life history of the plant, its variations as a whole, or the modifications of its parts under varying environmental conditions, may be added to the museum or other general collections.

3. Sources of material. Aside from the obvious sources of material for morphological and still more for histological study (such as the wild seed plants of the region, gardens, and greenhouses) there are others not less valuable. A very large number of admirable subjects for histological work can be had from the wholesale druggists, particularly those who make a specialty of "botanical" remedies. Almost any part of the plant body may be used in medicine, and many officinal substances, such as calisaya bark, the young wood of *Solanum dulcamara*, the rootstock of *Acorus*, the fruits of many *Umbelliferæ*, and a great number of other substances, to be had of dealers in drugs, form excellent material

for study. Seeds, bulbs, and rootstocks in great variety can be had of the seedsmen, and much useful material may be found among the fruits and vegetables sold by marketmen or provision dealers all the year round. Dealers in fine lumber and cabinet woods can supply many kinds which are of histological interest.

4. Collection of material at the proper season. In many cases the usefulness of material depends largely on its being collected at just the right stage of maturity or at some particular season. Experience will show the instructor at what time he can in his special locality best lay in the stock for his year's work. A few examples only need here be mentioned. Green corn (best of the large "dent" variety), just passing out of the milky condition, should be collected and boiled in water for about twenty minutes. It is then to be sliced from the cob, cutting deeply enough to preserve the embryos uninjured. The grains thus prepared may be preserved in alcohol indefinitely for study of the entire embryo.

Bean fruits of all ages, from the newly fertilized pistil to the fully developed but not dry pod, should be collected in season and put in preservative fluid, such as formalin, to illustrate the development of a fruit. If convenient, series of stages in the development of other fruits, e.g. apple, strawberry, orange, should be secured.

Indian corn plants should be dug up at various stages of growth and the lower part of the stem, with the attached roots, dried and preserved, after being thoroughly washed. These will illustrate secondary roots and successive steps in the formation of aërial roots. Pieces of corn stem of various ages show the development of bundles and of the very hard sclerenchyma of the rind.

Bits of *Aristolochia* or other stem used to illustrate open bundles should be collected in early summer, as soon as these are found to be well developed. A series of such stems cut at short intervals throughout the growing season is valuable.

Leaves of deciduous trees or shrubs should be collected as soon as fully grown and again when about to fall, and preserved in alcohol for the study of translocation of starch before falling.

Series of herbarium sheets and of specimens in preservative fluid should be secured to show the seasonal cycle of such plants as *Erythronium*, *Sanguinaria*, and many similar forms.

5. Field work. Many botanical topics can best be taught and some can only be taught in the field. It may be found desirable to interweave

instruction in ecological topics during field trips primarily undertaken for other purposes. So much depends on the nature of the region in which the work is done, the maturity of the class, the amount of time available for out-of-door study, and other circumstances, that only a few general principles for field work are here suggested.

(*a*) Every expedition should have one definite purpose. If many incidental subjects come up, they must not interfere with the primary object of the trip.

(*b*) All observations must be exact. If it is desired to ascertain the composition of a plant formation, the number of species and individuals occurring in measured units of area should be ascertained by actual count.

Temperatures of air, soil, or water should be noted with a good thermometer.

Illumination (in shaded areas) should be measured at several periods, e.g. two hours after sunrise, noon, and two hours before sunset.

In some cases it may be practicable to determine the relative moisture of the soil of several stations for at least the driest period of the year. Such results should be kept on record and may be taken into account by all classes which thereafter discuss the vegetation of those areas.

(*c*) The distribution of vegetation forms must be studied with reference to extreme conditions. Xerophytic structure does not always serve as a protection against a low annual rainfall but rather against severe periods of drought.

(*d*) Reasoning on ecological topics should be conducted with great caution. It is not safe to assume that attractiveness in any part of the plant body, as in the sweet cambium layer of the white pine or the inner bark of the slippery elm, is always of use to the plant. It may be highly disadvantageous. On the other hand, such "defenses" as the thorns on the trunk of the honey locust tree are of little or no conceivable use (on the branches they may be advantageous).

6. The study of Spirogyra. It is important to proceed slowly when students begin to use the compound microscope, and to make sure that correct habits of work are formed. The power to visualize or interpret objects studied with the compound microscope should be carefully trained. Simple models may be constructed. Thus the structure of the cell of *Spirogyra* may be demonstrated very effectively with a glass jar to represent the cell wall, a paper bag for the living plasma membrane,

strips of paper properly wound inside to represent the chromatophore, and some object suspended in the center to illustrate the position of the nucleus. A simple model of this character made with the coöperation of the class will greatly assist the students to a clear understanding of the study outlined in Sec. 56.

Whenever possible, a study of the *Amœba* or *Euglena* in comparison with the cell of *Spirogyra* will be found very helpful in making clear the characteristics of a protoplast.

Stained preparations of *Spirogyra* filaments (Sec. 211) may help to an understanding of the cell structure, but in general it is better that the student handle living material and with the aid of a few simple reagents (such as iodine and the salt solution) discover the facts for himself.

7. Cultures of Amœba. Cultures of *Amœba* may be made in an aquarium jar overstocked with filamentous algæ. Introduce sediment from a pool of *Oscillatoria* with a mass of the algæ; growths of *Scenedesmus* are also likely to have *Amabæ*. Place the jar near a window but just out of the direct rays of the sun. The filamentous algæ will decay and after a few weeks a coating will be formed on the sides of the jar. From this coating or from the sediment on the bottom of the jar *Amœbæ* can usually be obtained in quantity. They are generally most abundant on the sides nearest the light. In preparing material for the class gather a quantity of the slime and sediment with a pipette and place in a small bottle. After a few hours the water at the top will frequently contain so many *Amœbæ* that the students may readily find them in mounted drops.

8. Nuclear and cell division. Nuclear and cell division may also be studied from preparations of the pollen mother cells of the lily (Sec. 141) and in sections of various growing tissues such as stem tips, developing ovules, embryo sacs of lily, etc.

9. Cultures of Euglena. A culture of *Euglena* in a shallow dish with leaves and sediment at the bottom may be allowed to slowly dry up by evaporation. The *Euglenæ* will pass into the encysted condition. This dish of dried material may then be placed aside and will keep indefinitely. If water be added, the encysted *Euglenæ* will come forth again in the motile condition.

10. Sphærella and Volvox. If stones are placed in a dish swarming with *Sphærella*, the motile cells will settle upon them and pass into a

resting stage in which the cells develop heavy walls and the contents become reddish. Such stones may be dried, and when placed again in rain water will develop a new generation of green motile cells. Cultures may thus be carried along from season to season. The oöspores of *Volvox* will fall to the bottom of an aquarium among the sediment and after a time produce a new generation of *Volvox* colonies.

11. Hydrodictyon. *Hydrodictyon* may be readily grown in aquaria, where it will form successive generations of nets. Material cultivated in diluted Knop's solution, one half to one per cent (Sec. 200, A), is likely to form zoöspores after a few hours if transferred to pond or tap water. Nets placed in a five per cent solution of cane sugar (Sec. 200, C) are likely to produce gametes in a few days.

12. Coleochæte. *Coleochæte* frequently appears in aquaria, forming green disks on the sides of the glass. These may readily be detached with a scalpel and gathered with a pipette, and show the vegetative structure of the plant excellently. We have never known the alga to produce sexual fruit in aquaria.

13. Diatoms. The shells of diatoms in polishing powders and earths, or masses of fresh material, may be cleaned as follows. Heat in a small porcelain evaporator with c.p. sulphuric acid and slowly drop in a few crystals of sodium nitrate. After cooling rinse the sediment thoroughly in water, decanting frequently. It may then be dried on a slide and mounted permanently in balsam.

14. Bacteria. It is much more important that the bacteria be studied for the appearance of the growths *en masse* than that detailed microscopical examinations be made of these minute organisms. The microscopical examination of the "fur" from the teeth gives one of the most striking assemblages of forms. The cultures are best made by groups of students working with a set of Petri dishes in common. Excellent cultures of bacteria may be obtained in Petri dishes on potato agar as a substratum (Sec. 203), and these are preferable to cultures on slices of potato, but the preparation of agar requires more time and considerable laboratory equipment.

15. The bread mold. Individual cultures of bread mold may be readily made by the students and are more convenient for study than a large culture in common. Place small pieces of bread in watch glasses and set the latter over wet blotting paper in saucers, covering each with a tumbler. To make sure of good growths inoculate the pieces of bread

with spores from an old culture. These small cultures can be readily handled and studied with the hand lens, especially portions of the mycelium which may grow out over the surface of the watch glass. The bread mold is almost invariably followed by extensive growths of the green mildew, *Penicillium*.

16. Lichens. When time is limited it is more important that the lichen be studied than such *Ascomycetes* as *Microsphæra* or *Peziza*, since most lichens show excellently the fruiting characters of the sac fungi. The remarkable associations of fungi and algæ to form these composite organisms gives the lichens peculiar interest. A variety of lichens should be collected, dried, and fastened to cards for comparative studies of growth habits and form.

17. Gill fungi. The study of gill fungi must frequently be made at times inconvenient or impossible for field trips. The laboratory work may then be upon material preserved in strong alcohol or on edible forms sold in the markets (the commonest is *Agaricus campestris*). Horse manure placed under a bell jar will give excellent material of some small and delicate species of *Coprinus*, the development of which will prove interesting.

18. Diagrams and formulæ illustrating alternation of generations. The difficult subject of alternation of generations may generally be made clearer if the chief phases in the life histories of the types studied are represented by a series of diagrams. It is helpful if the diagrams are drawn with colored pencils; thus the gametophyte phases may be represented in yellow and the sporophyte phases in green. Life-history formulæ may be constructed after the manner suggested in the *Principles*, pp. 222, 278, 319, 336, and 375.

19. Mosses. Field studies of the mosses may not be possible at the time the type is studied in the laboratory. However, dried material is generally very satisfactory for habit studies on a variety of forms, and the greenhouses and cultures (Sec. 207) may be depended upon to furnish living material. Moss spores will generally germinate in sweetened water.

20. Ferns. A variety of ferns may be easily cultivated in the laboratory, which with herbarium material will give a very good idea of different forms of stems and leaves. A simple study of stem structure may be made by cutting across stems and whittling them lengthwise. Such an examination will make clear at least the position and importance

of the rigid tissue and fibro-vascular bundles. Fern spores may be germinated in sweetened water.

21. The pine. The pine is one of the best types of seed plants, easily available, to make clear the relationships between pteridophytes and spermatophytes. The general homologies between the staminate cone and the cones of the club mosses and horsetails are easily understood, as well as the homologies between the pollen grain with its inclosed male gametophyte and the microspores of pteridophytes with their male prothallia. The female gametophyte of the pine, also, is readily compared with the reduced female prothallia of heterosporous pteridophytes. For these reasons the life history of the pine is more easily understood with reference to the life histories of pteridophytes than is the life history of an angiosperm (such as the lily) where the gametophytes are much more reduced in structure.

The pine is also excellent for the study of the tissues of a tree with annual growth from a cambium ring. The structure of pine wood is one of the best exercises in the interpretation of cell structure, and the study outlined in Sec. 138, C, 5, is often given as a laboratory problem.

In a course outlining the evolution of life histories in plants the pine is as important a type as *Selaginella*, the fern, or the moss, besides having in itself a remarkably interesting morphology in relation to peculiar life habits.

22. The lily. There are perhaps no types more convenient than those of the lily family for the study of the gametophytes of the angiosperms. It is, however, not easy to present the life history of angiosperms in full from the study of a single type in a general course. It is probably better to use various forms which may be especially favorable for particular phases ; as, for example, the lily for the development of the pollen, but the elder for the male gametophyte ; the lily for the development of the embryo sac together with fertilization, double fertilization, and the origin of the endosperm, but the shepherd's purse for the development of the ovule and embryo.

23. The shepherd's purse. This plant would be almost perfect for a complete study of an angiosperm life history were it not for the small size of the flowers, anthers, and pistil, and in consequence the minuteness of the gametophytes. However, the shepherd's purse is one of the best types for the study of floral development (notwithstanding certain irregularities) and the development of the ovule and embryo.

GLOSSARY[1]

Actinomorphic (ray shaped). Having star-like or radiating symmetry.

Æcidium. A fructification peculiar to certain rusts producing æcidiospores, — a cluster cup.

Alternation of generations. The alternation in a life history of a sexual generation or *gametophyte* with an asexual generation or *sporophyte*.

Anemophilous (wind loving). A term applied to plants which are pollinated by the wind.

Angiosperms (vessel seed). Plants which have the seeds inclosed in an ovary or seed case.

Anther (flowering). The part of a stamen which bears pollen.

Antheridiophore (antheridia bearer). In the liverworts a specialized receptacle bearing antheridia.

Antheridium. The male sexual organ producing sperms or antherozoids in the groups below the seed plants; also called an antherid.

Antherozoid. See Sperm.

Antipodal (against the foot). The term applied to three cells at the base of the embryo sac.

Apical. Pertaining to the apex or tip.

Apical cell. A terminal cell which constitutes a growing point.

Apogamy. The development of an egg without fertilization, or the development of a sporophyte generation as a bud-like outgrowth from the gametophyte.

Apospory. The suppression of spore formation and the development of a gametophyte generation directly from the sporophyte.

Apothecium. In sac fungi, *Ascomycetes* (including lichens), the open cup- or saucer-shaped fructification in which the sacs or asci lie exposed in a membrane or hymenium.

Archegoniophore (archegonia bearer). In the liverworts a specialized receptacle bearing archegonia.

Archegonium (beginning of offspring). The many-celled female sexual organ producing an egg, characteristic of the bryophytes, pteridophytes, and some gymnosperms.

Archesporium (beginning of a spore). The cell or cells constituting the tissue from which the spores of bryophytes and pteridophytes are ultimately derived and also their homologues, the pollen grains.

[1] Most of the nouns in this glossary form their plurals by the addition of *s* or *es*, like ordinary English nouns. Those ending in *us*, unless otherwise stated, form the plural in *i*, as *nucleus*, plu. *nuclei*. Those ending in *ium* form the plural in *ia*, as *antheridium*, plu. *antheridia*.

Ascocarp (sac fruit). The fructification in which asci are formed.
Ascogonium (sac offspring). The female sexual organ of the sac fungi, or *Ascomycetes*.
Ascospores (sac spores). The spores developed in the ascus.
Ascus (a sac). One of the spore-producing cells of an ascocarp; each ascus generally develops eight spores.
Asexual spore. One having no immediate relation to sexual cell unions.
Association. One of the ecological unit groups (smaller than a plant *formation*) of which the formation is sometimes made up.
Axial. Concerning or belonging to the axis.
Axil (the armpit). The upper angle formed by the junction of the leaf and stem.
Axis (an axle). An imaginary central line about which organs are developed or ranged.

Basidium (a little pedestal). A spore-bearing cell in the basidia fungi.
Bast, hard. The fibrous portion of the phloëm.
Bast, soft. The portion of the phloëm composed of sieve tubes.
Bilateral symmetry. The arrangement of parts in corresponding right and left halves, — zygomorphism.
Bisexual. Term used of flowers having both stamens and pistils.
Bract. A modified leaf of an inflorescence.
Bryophytes (moss plants). The great group composed of the liverworts and mosses.
Bulb. A short subterranean stem or bud with fleshy scales.

Calyptra (a veil). The cap covering the developing spore case of a moss (and also a liverwort), formed by the enlargement of the archegonium after fertilization.
Calyx (a cup). A collective term for the sepals or outer members of the perianth.
Cambium (to change). The meristematic layer which in dicotyledons lies between the xylem and phloëm parts of each fibro-vascular bundle and forms a very thin cylinder joining wood and bark.
Canal cells. A row of cells in the neck of an archegonium above the egg.
Capsule (a box). A dry, dehiscent seed vessel.
Carpel (fruit). The simplest form of seed-bearing organ; a simple pistil or one of the parts of a compound pistil; morphologically a *megasporophyll*.
Carpogonium (fruit offspring). The female sexual organ of the red algæ.
Carpospore (fruit spore). A spore developed in the cystocarp of a red alga.
Cell (a small chamber). A unit of protoplasm; a *protoplast* (with or without a cell wall).
Cell wall. The carbohydrate membrane, generally cellulose, by which plant protoplasts are usually inclosed.

Cellulose. The carbohydrate material of which cell walls are formed.

Central cylinder. The stele or portion of a root or stem which is inclosed by the primary cortex.

Chlamydospore (cloaked spore). A thick-walled resting cell or spore.

Chlorophyll (leaf green). The ordinary green coloring matter of plants held in the chloroplasts, or chromatophores.

Chlorophyll bodies. Masses of protoplasm (in seed plants usually minute disk-shaped bodies) colored green by chlorophyll, — chloroplasts.

Chloroplasts (green molded). Chlorophyll bodies; plastids containing chlorophyll.

Choripetalous (separate petal). Having the petals separate.

Chorisepalous (separate sepal). Having the sepals separate.

Chromatin (color). The deeply staining substance contained in the nucleus which forms the chromosomes.

Chromatophore (color bearer). Any large green, brown, or red protoplasmic body, especially characteristic of the cells of algæ.

Chromoplast (color molded). Plastids of other colors than green (as red, brown, yellow, etc.), a term used in contrast to chloroplast.

Chromosomes (color bodies). Readily stained bodies within the nucleus, composed of chromatin and appearing most conspicuously during nuclear division.

Cilium (an eyelash). A vibrating fibril attached to a zoöspore or sperm.

Cladophyll (branch leaf). A branch with the form and functions of a leaf, called also a cladode and phylloclade.

Class. A taxonomic group composed of orders.

Cleistogamous (closed marriage). A term applied to fertilization occurring in unopened flowers.

Closed bundle. A fibro-vascular bundle which contains no cambium and is consequently incapable of further growth.

Cœnocyte (a vessel in common). A multinucleate cell, generally of large size.

Cœnogamete (a gamete in common). A multinucleate gamete, generally of large size.

Collenchyma. Parenchyma cells with walls thickened, usually at the angles.

Columella, plu. *columellæ* (a small column). The persistent axis of certain spore cases and spore fruits.

Companion cell. An elongated cell associated with a sieve tube.

Conceptacle. A pit-like cavity in the rockweeds containing the sexual organs.

Cone. The scaly fruit of such conifers as the pines, spruces, etc., also of the lycopods and horsetails, — a strobilus.

Conidiophore (conidia bearer). A generally upright stalk upon which conidia are borne.

Conidium (dust). An asexual spore of a fungus, generally formed in the air.

Coniferous. Cone bearing.

Conjugation. The sexual union of similar gametes to form a *zygospore* or zygote.
Cork. Protective tissue in the outer portions of the bark.
Corm (a trunk). The bulb-like fleshy base of some stems.
Corolla (a small crown). A collective term for the petals or inner members of the perianth.
Cortex. The bark or rind.
Cotyledon. An embryo leaf borne by the hypocotyl.
Cuticle (the outer skin). The exterior layer of the epidermis.
Cystocarp (bladder fruit). The fruit of the red algæ resulting from the fertilization of the carpogonium.
Cytoplasm (cell plasm). The general protoplasm of the cell exclusive of the nucleus and plastids.

Deciduous (to fall). Falling when their function is performed, as the leaves of most hard-wood trees in temperate climates.
Dehiscent (to yawn). Opening spontaneously when mature, as anthers, to discharge pollen, or as capsules, to discharge spores.
Dermatogen (skin producer). The layer of cells around growing points from which the epidermis is derived.
Determinate. A term applied to stems where the growth in length is determined by the presence of a winter bud, and to an inflorescence where there is a terminal flower bud.
Diageotropic. Growing horizontally under the influence of gravity, as some branches do.
Dicotyledonous. Having two seed leaves or cotyledons.
Dimorphous flowers (two forms). Flowers which have two forms, as long and short styled.
Diœcious (two households). Unisexual, the male and female sexual organs borne by separate individuals.
Dorsiventral (back, belly). Having upper and lower faces, as in most leaves.
Drupe (an olive). A stone fruit, as a peach or plum.

Ecology (household discourse). The study of plants in relation to their surroundings. The term is often made to include much of the subject-matter of ecological plant geography, or the distribution of plants on the earth with reference to environment. In this sense it is very frequently used in connection with the study of plant formations.
Egg. A nonmotile female gamete, generally large in comparison with the sperm.
Egg apparatus. A group of three cells at the micropylar end of the embryo sac, consisting of the egg and two synergids.
Elater (a driver). A spirally thickened elongated cell or other filamentous structure developed to assist in expelling spores from a spore case.

Embryo (a rudimentary animal). The rudimentary plantlet, as in the seed.

Embryo sac. The cavity which contains the female gametophyte of a seed plant and later the embryo and endosperm (if present) in the seed.

Emergence. An outgrowth from the surface of a plant not (like a hair) arising solely from the epidermis nor (like a thorn) from a bud.

Endosperm (within the seed). A parenchymatous tissue formed within the embryo sac and often developed into the principal mass of reserve material in the seed.

Entomophilous (insect loving). A term applied to plants that are pollinated by insects.

Enzyme (in yeast). An unorganized or soluble ferment (such as diastase) which is not associated with any organism.

Epidermis (upon the skin). The cellular skin or covering of the plant body inside the cuticle.

Epigynous. A term applied to flowers in which the stamens and perianth appear to grow from the top of the ovary.

Epiphyte (upon a plant). A plant which grows upon other plants but not parasitically, — an air plant.

Eusporangiate. A term applied to pteridophytes the sporangia of which arise from a group of cells.

Family. A taxonomic group standing between genus and order.

Fertilization. The fusion of two sexual cells, especially the fusion of the sperm with the egg.

Fiber. A slender, thick-walled cell, many times longer than its width.

Fibro-vascular. Composed of fibers and vessels, as a fibro-vascular bundle.

Filament (a thread). The stalk of a stamen bearing the anther.

Fission. The process of cell division by a gradual pinching in two of the cell.

Flower. An assemblage of organs in the seed plants necessary for fertilization, often with protecting envelopes. The flower of the angiosperms when bisexual usually consists of a perianth, stamens, and pistil or pistils.

Foot. A portion of the sporophyte set apart to absorb water or nourishment from the gametophyte.

Formation. An ecological term denoting a well-defined assemblage of plants characteristic of a given kind of station, as a peat bog.

Frond (a leaf). The leaf of a fern, generally both vegetative and spore producing in its functions.

Fruit. The ripened seed case and its contents, or, in a broader sense, a spore-producing structure of the lower plants.

Fundamental tissue. The general ground tissue (mostly undifferentiated) in which fibro-vascular bundles and other specialized tissues arise.

Funiculus (a little rope). The stalk by which the ovule or seed is attached to the placenta.

Gametangium (gamete vessel). The organ which produces gametes.
Gamete. A sexual reproductive cell which ordinarily must fuse with another gamete in order to live.
Gametophyte (gamete plant). The sexual plant in an alternation of generations, producing sexual cells or gametes (see Sporophyte).
Gemma, plu. *gemmæ* (a bud). An asexual reproductive body, generally many celled, rather characteristic of the bryophytes.
Generative cell. The cell within the male gametophyte of seed plants from which the two sperm nuclei are developed.
Genus, plu. *genera* (a race). The taxonomic group composed of related species.
Geotropism (earth turning). The action of gravity in directing growth.
Gill. A flat spore-bearing plate on the under side of a mushroom or toadstool.
Glume (a husk). A chaffy bract on the inflorescence of grasses.
Grain. Such a seed-like fruit as that of the grasses,—a minute, roundish body, as a starch grain.
Ground tissue. See fundamental tissue.
Growing point. The meristematic tip of the root or stem from which the tissues are produced.
Guard cell. One of the cells (usually two in number) which serve to open and close a stoma.
Gymnosperms (naked seeds). Plants (as the *Coniferæ*) which have no closed ovaries, so that the seeds are borne naked, usually on scales.

Halophyte (salt plant). A plant which habitually grows in saline soils, as on sea beaches or in salt marshes.
Haustorium (a drawer). A sucker-like absorbing organ of a parasitic plant.
Heliotropism (sun turning). The action of light in directing growth.
Hermaphrodite. Having both forms of sexual organs together in the same structure; bisexual.
Heterocyst (unlike cell). In the blue-green algæ a large cell, empty or almost empty of protoplasm.
Heterogamy (unlike gametes). The condition where the pairing gametes are different in form and structure, as the egg and sperm.
Heterospory (unlike spores). The condition in which a sporophyte produces spores of two sizes, microspores and megaspores.
Hilum. The scar on a seed showing its point of attachment to the funiculus or the placenta.
Holdfast. An organ of attachment developed by certain algæ.
Homologous (similar discourse). Of one type, though differing in form and function.
Homospory (similar spores). The condition in which a sporophyte produces spores of the same size.
Hormogonium (chain offspring). A portion of the filament of a blue-green alga reproductive in function.

Host. A plant or animal which nourishes a parasite.

Hybrid (a mongrel). The offspring obtained by the action of the pollen of one species on the pistil of another. The term is also used for the offspring of any cross.

Hydrophyte (water plant). A water plant.

Hygroscopic (moisture seeing). Expanding or shrinking readily under the influence of moisture.

Hymenium (a membrane). An expanded fruiting surface of a fungus.

Hypha, plu. *hyphæ* (a web). The filament of a fungus.

Hypocotyl. The portion of an embryo or very young seedling between the cotyledons and the root.

Hypogynous. A term applied to flowers in which the stamens and perianth grow from beneath the ovary.

Indeterminate. A term applied to stems where the growth in length is indefinite because no terminal bud is formed, and to an inflorescence where there is no terminal flower.

Indusium. In ferns a protective outgrowth from the leaf covering a cluster of sporangia or sorus.

Inferior ovary. See Epigynous.

Inflorescence (flowering). The manner in which the flowers are arranged in the flower cluster.

Intercellular. Between the cells or among them.

Internode. The portion of the stem between two nodes.

Involucre (a wrapper). A ring of bracts surrounding several flowers or their flower stalks.

Irritability (easily excited). Sensitiveness to stimuli, such as light, heat, gravity, etc.

Isogamy (equal gametes). The condition where the pairing gametes are similar in form and structure.

Lamina, plu. *laminæ* (a layer). The blade of a leaf.

Leaf trace. The group of fibro-vascular bundles which connects the veins of the leaf with the fibro-vascular system of the stem.

Lenticel. A roundish or lens-shaped spot on young bark, marking the former position of a stoma.

Leptosporangiate. A term applied to pteridophytes in which the sporangium arises from a single epidermal cell.

Leucoplast (white molded). A protoplasmic body found in cells in the interior of the plant body, often serving as a starch builder, — a colorless plastid.

Lignin (wood). The thickening material deposited in cell walls to produce woody tissue.

Locule (a little compartment). A cavity or chamber, as of an ovary.

Medullary (belonging to the marrow). Related to the pith, as the medullary rays.

Megasporangium (large spore vessel). The sporangium which develops megaspores.

Megaspore (large spore). The larger one of the two sorts of spores produced by heterosporous pteridophytes; it gives rise to a female gametophyte.

Megasporophyll (large spore leaf). A leaf bearing megaspores.

Meristem (divisible). Formative, rapidly dividing tissue such as cambium or the cells of growing points.

Mesophyll (middle leaf). The entire parenchyma of the leaf, inside the epidermis.

Mesophyte. A plant adapted to live with a moderate amount of soil water and humidity.

Micropyle (small gate). The small opening between the integuments leading to the nucellus of an ovule.

Microsporangium (small spore vessel). A sporangium which develops microspores.

Microspore (small spore). The smaller of the two sorts of spores produced by heterosporous pteridophytes; it gives rise to a male gametophyte.

Microsporophyll (small spore leaf). A leaf bearing microspores.

Mitosis (a thread or web). The process of indirect nuclear division characterized by the presence of a spindle.

Monocotyledonous. Having only one seed leaf or cotyledon.

Monœcious (one household). Having the male and female sexual organs borne separately by the same individual.

Morphology (form discourse). The science of the form and structure of an organism.

Mutation. A decided and abrupt departure in the offspring from the characters of the parent, often sufficient to constitute a new species.

Mycelium (fungus growth). A mass of vegetative fungal filaments, or hyphæ.

Nastic. A term applied to movements produced by all-round (not one-sided) stimuli. The opening and closing of such flowers as the crocus, tulip, etc., are thermonastic movements.

Nectary. The organ in which nectar is secreted.

Node (a knot). The part of a stem which normally bears a leaf or group of leaves.

Nucellus (a little kernel). The portion of the ovule within the integuments and containing the embryo sac.

Nucleolus (diminutive of nucleus). A small readily stained body, generally present with the chromatin, in the nucleus; also called a nucleole.

Nucleus (a kernel). The organ of the cell containing the chromatin and nucleolus.

Oögonium (egg offspring). The cell in the thallophytes which develops the egg; also called an oögone.

Oösphere (egg sphere). An egg cell.
Oöspore (egg spore). A fertilized egg which develops a heavy wall and passes through a period of rest before germinating.
Open bundle. A fibro-vascular bundle which contains cambium and is consequently capable of further growth.
Operculum, plu. *opercula* (a cover). In mosses the cover of the spore case.
Order. A taxonomic group composed of families.
Osmosis (a thrusting). The diffusion or interchange of liquids through membranes.
Ovary. The ovule-bearing part of the pistil.
Ovule. The undeveloped structure which after fertilization becomes the seed.

Palisade cells. Elongated parenchyma cells of a leaf, which lie beneath the epidermis with their long axes at right angles to the leaf surface.
Palmate (like the palm of the hand). With veins or sinuses radiating like fingers.
Parasite. An animal or plant that obtains its food from some other living organism, called its host.
Parenchyma. Tissue composed of nearly globular cells or polyhedral cells the diameters of which are approximately equal, as pith.
Parietal (a house wall). Pertaining to a wall, as a placenta on an ovary wall.
Parthenogenesis (virgin generation). The development of an egg or other gamete without the process of fertilization.
Pathogenic (disease offspring). Producing disease.
Pedicel (a little foot). The stalk on which an organ is borne, especially the flower stalk of each separate flower in a cluster.
Peduncle (a little foot). The flower stalk.
Perianth (around the flower). A collective term for calyx and corolla taken together.
Periblem (clothing). The part of the meristem at the growing apex of a root or shoot, immediately beneath the epidermis. It develops into the cortex.
Pericambium. See Pericycle.
Pericycle. The outermost layer of the central cylinder of a root.
Perigynous. A term applied to those flowers in which the stamens and perianth appear to grow from around the wall of the ovary.
Peristome (around the mouth). In mosses the circle of teeth or segments surrounding the opening of the spore case.
Perithecium (around a case). In sac fungi, *Ascomycetes* (including lichens), a cavity containing the sacs or asci.
Petal (a flower leaf). A leaf of the corolla.
Petiole (a little foot). A leaf stalk.
Phloëm (bark). The soft portion of a fibro-vascular bundle, — the bast. In dicotyledons the part outside of the cambium, — the inner bark.
Photosynthesis (light putting together). The process of manufacture of carbohydrates, such as starch and sugar, from water and carbon

dioxide. It is carried on by the chromatophores and chloroplasts acted on by the energy of sunlight.

Physiology. The science of the action and functions of organisms.

Pinnate (a feather). Having leaflets arranged along two sides of a main leaf axis.

Pistil (a pestle). The simple or compound structure (composed of one or more carpels) which in angiosperms contains the ovules.

Placenta. The ovule-bearing portion of the interior of the ovary.

Plageotropic (oblique turning). Assuming an oblique direction under the influence of gravity, as most secondary roots.

Plasma membrane. The limiting membrane of a protoplast.

Plasmolysis (that which is formed, loosing). A separation by osmotic action of the protoplast from the cell wall.

Plastid (that formed). A protoplasmic body usually with a special function. The term is used collectively for chloroplasts, chromoplasts, and leucoplasts.

Plerome (that which fills). That part of the meristem near a growing point which is surrounded by the periblem and develops into the central cylinder.

Plumule (a little feather). The primary leaf bud of an embryo seed plant.

Pod. A dry, many-seeded, dehiscent fruit.

Pollen (fine flour). Minute grains developed in the pollen mother cells of the anther and essential for the fertilization of the ovule. The locules of the anther are morphologically microsporangia and the pollen grains are microspores.

Pollen tube. The structure which is developed from the inner coat of the pollen grain and serves to carry the sperm nuclei into the embryo sac of the ovule.

Pollination. The transference of the pollen to the stigma or to the naked ovule of the gymnosperms.

Prosenchyma. Tissue composed of elongated cells.

Proteid. Any one of a group of nitrogenous compounds of which albumen is an example.

Prothallium (before a young shoot). The gametophyte developed from the spore of a pteridophyte.

Protonema, plu. *protonemata* (first thread). A filamentous growth developed from the spore of a moss, from which the leafy moss plants arise.

Protoplasm (first formed). The living part of the material of the plant or animal body contained in the cells.

Protoplast. A unit of protoplasm, or cell, with or without a cell wall.

Pteridophytes (fern plants). The great group composed of the ferns, horsetails, and club mosses.

Pyrenoid (resembling a kernel). Minute bodies imbedded in the chromatophores, which act as centers of starch formation.

Receptacle. The extremity of the flower stalk, on which the floral parts are borne; in *Compositæ* the common receptacle bears the head of flowers, — any structure carrying sexual organs.

Rhizoid (resembling a root). A root-like filament in the lower plants.

Rootstock. A somewhat root-like stem, usually nearly horizontal and dorsiventral, extending either above or under ground.

Saprophyte (rotten plant). A plant that lives on dead organic matter.

Scape (a stem). A leafless peduncle arising from the ground.

Sclereid (hard). See Stone cell.

Sclerenchyma. Rigid or strengthening tissue, composed of thick-walled cells, often having the form of fibers.

Secondary growth. The growth which takes place in gymnosperms and woody dicotyledons from the development of the cambium cylinder.

Seed. The fertilized and matured ovule.

Seed plant. A member of the highest division of the plant kingdom, characterized by producing seeds.

Sepal (a covering). A leaf of the calyx.

Sieve tubes, or **Sieve cells.** Soft bast or phloëm cells with perforated *sieve plates* in their walls.

Species. A kind of plant or animal, one of the taxonomic subdivisions of a genus.

Sperm. A male gamete, generally very small and motile in comparison with the egg.

Spermatia. Non-motile sperms, as in the red algæ.

Spermatophytes (seed plants). The great group composed of seed plants.

Spermogonium. In the rusts a cup-shaped receptacle producing minute cells (spermatia) believed to be sperms no longer functional.

Spindle. A mechanism consisting of delicate fibrils concerned with the distribution of the chromosomes during nuclear division (mitosis).

Sporangium (spore vessel). A spore-producing case.

Spore (seed). A term applied to a variety of one- or few-celled reproductive bodies characteristic of groups below the seed plants.

Sporidium (diminutive of spore). A spore produced by a promycelium.

Sporogonium (spore offspring). The sporophyte generation of the liverworts and mosses, sometimes called the fruit.

Sporophyll (spore leaf). A leaf which bears spores.

Sporophyte (spore plant). The asexual plant in an alternation of generations producing asexual spores (see gametophyte).

Stamen. The pollen-bearing organ of seed plants; morphologically a *microsporophyll.*

Stele (a pillar). The central cylinder of a stem or root. Sometimes a stem has more than one plerome strand at the growing point and so develops several cylinders and is called *polystelic.*

Stigma (a spot or mark). The portion of the pistil (destitute of epidermis) on which the pollen lodges and germinates.

Stoma, plu. *stomata* (a mouth). An opening through the leaf epidermis which serves for transpiration. The *stomatic apparatus* consists of the stoma and its guard cells.

Stone cell. A hard cell with its walls much thickened by secondary deposits, as the grit cells of the pear.

Strobilus (a fir cone). A cone-like cluster of sporophylls.

Style (a pillar). An elongation of the pistil above the ovary, bearing the stigma.

Suspensor. In seed plants and club mosses a structure arising from the fertilized egg, which pushes the developing embryo deep into the tissue of the gametophyte or endosperm.

Symbiont. An organism living in a condition of symbiosis.

Symbiosis (living together). The condition in which two or more organisms are living in intimate physiological relationship.

Sympetalous. With the petals appearing as if grown together by their edges.

Synergids (co-workers). Two cells accompanying the egg at the micropylar end of the embryo sac, the group of three constituting the egg apparatus.

Synsepalous. With the sepals appearing as if grown together by their edges.

Taxonomy (order, law). The study of classification. Plant taxonomy is often called systematic botany.

Teleutospores (end spores). The resting spores (chlamydospores) of the rusts, producing a promycelium.

Testa (a shell). The outer coat of the seed.

Tetraspore (four spore). An asexual spore characteristic of the red algæ, usually produced in groups of four.

Thallophytes (thallus plants). The great group composed of the algæ and fungi.

Thallus (a young shoot). A simple vegetative body without differentiation into roots, stem, or leaves.

Tissue. A definite region of similar cells with the same functions.

Trachea, plu. *trachea* (the windpipe). See Vessel.

Tracheid (trachea-like). An elongated cell with closed ends and the walls with secondary thickening, as the pitted cells of coniferous wood.

Trichogyne. A delicate filamentous extension from the carpogonium, specialized to receive the sperms.

Tropophyte. A plant which is mesophytic during part of the year and xerophytic during the remaining part, as most deciduous trees.

Turgor. The inflated or distended condition of a cell which is full of liquid.

Unisexual. Having only male or female reproductive organs.

Uredospore (blight spore). A spore of the rusts for rapid multiplication (summer spore).

Variety. A subdivision of a species.

Vein. A fibro-vascular bundle of a leaf, petal, or other thin and flat organ.

Venation. The manner in which veins are distributed.

Venter. The swollen basal portion of an archegonium containing the egg.

Vernation. The manner of unfolding in buds.

Vessel. A tube or duct made of separate sections but continuous from the absorption of the cross partitions. The walls have various thickening deposits, often spiral or ring-formed.

Volva (a wrapper). An envelope inclosing a young toadstool and ruptured by the growth of the latter, portions sometimes remaining as scales on the top of the cap and sometimes as a cup at the base of the stalk.

Xerophyte (dry plant). A plant which can live with a scanty supply of water.

Xylem (wood). The wood or inner part of a fibro-vascular bundle, the portion within the bundle cambium.

Zone. In ecology a band of any given plant formation, usually bounded by other bands representing other formations, as about a pond, a salt spring, etc.

Zoöspore (animal spore). A ciliated and therefore motile asexual spore.

Zygomorphism (yoke form). The arrangement of parts in corresponding right and left halves; bilateral symmetry.

Zygospore (yoke spore). A sexually formed spore resulting from the fusion of similar gametes (isogamy); also called a zygote.

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